

Title: Efficacy of Ultraviolet C disinfection for inactivating Senecavirus A on contaminated surfaces commonly found on swine farms – #18-114 IPPA

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Industry Summary:

Seneca virus A (SVA) is clinically indistinguishable from other vesicular diseases such as foot-and-mouth disease virus (FMDV). Since 2015, the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) has experienced an increased number of SVA positive swine cases and while SVA has little impact on production and is not a reportable disease, swine veterinarians in the United States are required to report animals exhibiting vesicular lesions to State or Federal animal health officials. The increased incidence of SVA has stressed state and federal resources to conduct FAD investigations and has reduced the sense of urgency when vesicular lesions are observed because of the assumption “it is just SVA”. Carrying agents that are either infected or contaminated with live virus may transmit SVA, as well as other swine pathogens, from one herd to another. One approach to reducing the risk of transmitting the virus is to mitigate the contamination of carrying agents after they become contaminated. Ultraviolet C (UVC) light disinfection has been applied in many industries including human medicine and food processing, but the practical application of the technology in livestock production has not been well studied. Despite the lack of research, the technology is increasingly being used on swine farms. UVC disinfection is potentially an economically feasible, easily managed, effective way to mitigate risk associated with the entry of carrying agents contaminated with SVA and other viruses.

The objective of this study is to evaluate the efficacy of UVC for inactivating Seneca virus A (SVA) on three different contaminated surfaces (cardboard, cloth and plastic) commonly found in swine farms under challenged conditions.

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The plastic coupons inoculated with SVA and PBS had a significantly lower virus log titer, (>7 log virus titer reduction) in both the PTC and SER when compared to their relative positive controls. All other study groups had only a 2 log virus titer reduction or lower in the PTC and SER compared to their positive controls. There was no significant statistical difference between the UVC top and bottom inoculated surfaces. UVC was efficacious as a disinfection for SVA on plastic that was free of organic material. UVC has the potential to serve as an appropriate method of disinfection for SVA on materials that are free of organic material and non-permeable, such as plastic.

Keywords: swine, biosecurity, ultraviolet C, Senecavirus A, disinfectants

Scientific Abstract:

The objective of this study is to evaluate the efficacy of ultraviolet C (UVC) for inactivating Seneca virus A (SVA) on three different contaminated surfaces (cardboard, cloth and plastic) commonly found in swine farms under challenged conditions.

An experimental design study under controlled conditions assessed the effect of UVC on a SVA isolate on three different surface types: cardboard, cloth and plastic. Each coupon was inoculated with 1 ml of SVA and 1 ml of PBS or 1 gram of feces on the top or bottom surface of the coupon and allowed to dry. Coupons were exposed to UVC in a commercially available pass through chamber (PTC) for 5 minutes or in a simulated supply entry room (SER) for 120 minutes. After exposure, virus was recovered from each coupon and virus titration was performed on the sample collected. Log reductions of the UVC treated groups were compared to their relative positive controls.

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Introduction

Seneca virus A (SVA) is a non-enveloped, single stranded RNA virus in the family *Picornaviridae* and is associated with vesicular lesions.¹ SVA is clinically indistinguishable from

other vesicular diseases such as foot-and-mouth disease virus (FMDV). Since 2015, the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) has experienced an increased number of SVA positive swine cases and while SVA has little impact on production and is not a reportable disease, swine veterinarians in the United States are required to report animals exhibiting vesicular lesions to State or Federal animal health officials. The increased incidence of SVA has stressed state and federal resources to conduct FAD investigations and has reduced the sense of urgency when vesicular lesions are observed because of the assumption “it is just SVA”. The reduced sense of urgency could be devastating in the event of an FMDV outbreak, because timely recognition is critical to minimize the spread. The increased incidence of SVA in the United States increases the likelihood that veterinarians will assume that the presence of vesicular lesions are due to SVA and not report them to State or Federal animal health officials or that animals arriving at first points-of-contact from premises previously diagnosed with SVA positive animals will be released for slaughter without further investigation, putting early recognition of FMDV at risk.

Carrying agents that are either infected or contaminated with live virus may transmit SVA, as well as other swine pathogens, from one herd to another. One approach to reducing the risk of transmitting the virus is to mitigate the contamination of carrying agents after they become contaminated. For SVA, relatively little research has been done to identify methods to inactivate the virus on contaminated surfaces. One study evaluated several disinfectants on aluminum, rubber, plastic, stainless steel and cured cement.² Chlorine bleach; a quaternary ammonium and glutaraldehyde combination (Synergize) and a phenol (Tektrol) were evaluated.

The disinfecting properties of short-wave ultraviolet light (UVC), with wavelengths from 200 to 280nm, are well known and ultraviolet disinfection is particularly effective for inactivating single-stranded RNA viruses, like SVA, on surfaces.³ The process in which UVC works is by the formation thymine, cytosine, or uracil dimers in the DNA or RNA of viruses causing mutations and essentially inactivating the virus.⁴ The technology has been applied in many industries including human medicine and food processing, but the practical application of the technology in livestock production has not been well studied. Despite the lack of research, the technology is increasingly being used on swine farms. Applications include treatment of incoming supplies, employee lunches and load-out areas. UVC disinfection is potentially an economically feasible, easily managed, effective way to mitigate risk associated with the entry of carrying agents contaminated with SVA and other viruses.

Objectives:

The objective of this study is to evaluate the efficacy of UVC for inactivating SVA on three different contaminated surfaces (cardboard, plastic and cloth) commonly found in swine farms under challenged conditions, such as in the presence of organic material and partial covering the contaminated coupon during UVC disinfection.

Material and Methods:

Experimental design

An experimental design study under controlled conditions assessed the effect of UVC on a characterized SVA isolate on three different surface types (plastic, cloth and cardboard) described in Table 1. These surfaces were evaluated under challenged conditions commonly seen

on swine farms such as in the presence of organic material (feces) or not, and direct exterior surface exposure vs partial covering of the contaminated exterior surface by the shelving units during UVC exposure. A total of 39 study groups were evaluated with three replicates per study group. Treatment groups included: “UVC Top” which was defined as exterior surface inoculation of the coupon surface facing up towards the top of the pass through chamber (PTC) or ceiling of the supply entry room (SER) with direct exposure and “UVC Bottom” which was defined as exterior surface inoculation of the coupon that would be face down and partially covered by the shelf in the PTC or SER. Each UVC Top and UVC Bottom groups had feces present or not present. Groups that had feces present were called “UVC Top or Bottom Feces”. There was a positive control group with and without feces and a negative control group for each coupon. Temperature and humidity were controlled at approximately 25°C and 40%, respectively. Coupons for each different surface type were manufactured to be 3 in diameter circles.

Virus Propagation

The SVA isolate USA/SD41901/2015 was initially obtained in swine testicular (ST, ATCC CRL-1746) cells from vesicular lesion swabs collected from a finishing pig in South Dakota in 2015. Briefly, the vesicular lesion swabs were processed in phosphate buffered saline (10%, wt/vol). After centrifugation at 4,200 x g for 10 min at 4°C, the supernatant was filtered (0.22 µM) and inoculated into semiconfluent ST cells in 24-well plates. After 1 hour of adsorption, the inoculum was removed and 1 milliliter (ml) of complete MEM medium was added to each well. Cells were incubated at 37°C with 5% CO₂ for 4-5 days. Development of cytopathic effect (CPE) was monitored. Isolation of SVA was confirmed by real-time RT-PCR and immunofluorescence assay. The SVA isolate had a titer of approximately $1 \times 10^{7.5}$ median tissue culture dose (TCID₅₀) per ml. This SVA isolate was further propagated in H1299 (ATCC CRL-5803) cells to obtain more volume for use in this study. Titration of the propagated SVA isolate were conducted in H1299 cells.

Surface Inoculation

One ml of stock virus ($3 \times 10^{7.5}$ median tissue culture dose (TCID₅₀) mixed with 1 mL of PBS or 1 gram (g) of feces (PEDV, PRRSV and SVA negative) was pipetted and spread onto the exterior surface of each coupon with care not to allow any liquid to runoff the edges of the coupon. The treatment groups with feces present had an additional 0.3 microliters of PBS added to the 1 g of feces and 1 mL of stock virus to create a liquid content that could be spread onto the exterior surfaces of the coupon. All coupons were allowed to air dry for approximately 90 minutes.

UVC units evaluated

The two devices utilized in this study are the Bioshift® Pass through Germicidal UV chamber (PTC) (Once®, Plymouth, MN) and the ESP-DLux UVC unit (Elevated Health Systems, Wichita, Kansas). The PTC measures 23 ½ inches long x 29 ¾ inches wide x 24 inches high. There are two openings to the chamber, one designated to be located on the “dirty side” of the farm and one designated to be on the “clean side”. The interior of the chamber was approximately 20 in x 20 in x 20 in. There are four 18 inch UVC bulbs located at each corner of the chamber that emit wavelengths of 254 nm. One metal wire was present within the chamber.

The wire gauge was approximately 2 mm thick. The shelf sat approximately 1 inch from the bottom of the UVC chamber. Safety features include an emergency stop, that will not operate unless both doors are shut, and the windows are protected by glass that is not transparent to UVC light for eye protection. The model can be seen in Figure 1.

The ESP-DLux UVC units emits UVC at the wavelength of 254 nm, and is non-ozone producing. An 8 foot (ft) by 8 ft by 8ft room was constructed to simulate a supply entry room (SER) that would have a similar set-up to the UVC PTC described above. Four ESP-DLux UVC units were present, with one centrally located between each corner of the room. An approximately 4 ft high metal wire shelf was centrally located in the SER (Figure 2). The walls and ceilings of the SER were painted with a reflective paint, Lumcept™, made specifically for UVC disinfectant devices (Lumaccept Inc).

UVC Exposure

The UVC exposure time for the PTC was 5 minutes based on the manufacturer's recommendations. The UVC exposure time for the SER was calculated based on the inverse square law which states that the amount of radiant exposure is inversely proportional to the square of the distance from the source, or as distance is doubled, radiant exposure (per unit of time) is reduced by ¼. Based on the equation, $\text{Dose (mJ/cm}^2\text{)} = \text{Intensity (mW/cm}^2\text{)} \times \text{Time (s)}$, a time exposure can be calculated if the intensity and dose of that particular agent is known. A maximum intensity of approximately 0.80 mW/cm² was recorded for the PTC at a distance of approximately 20 inches with an exposure time of 5 minutes (300 sec). It was calculated that the UVC SER exposure time should be approximately 120 minutes (7200 sec) based on reducing the intensity by a quarter each time the distance was doubled until reaching approximately 96 in (8ft) to obtain a UVC lethal dose greater than 41 mJ/cm².

UVC intensity (mW/cm²) was measured during UVC exposure with a commercially available UV254SD data logging UVC/UVA meter (General Tools and Instruments LLC, Secaucus, NJ) every 2 and 30 seconds for the PTC and SER, respectively.

Three coupons were placed in the center of the PTC, evenly spaced in a vertical row or in a 3 x 3 square on a shelf, centrally located between the ESP-Dlux bulbs, in the SER facing towards the ceiling or face down on the metal wire shelf, according to study group. To account for potential variation of UVC light angle in the PTC and SER each coupon replicate per UVC treatment group was placed in a different position in the replicate row within the PTC or SER so that every coupon would be exposed to UVC treatment at a different location in the row. The UVC meter was placed beside the coupon near the "clean" door for the PTC and in front of the last row, closest to the door in the SER, as shown in Figures 1 and 2, respectively. To avoid contamination of the shelves in both the PTB and SER, coupons were placed on top of sterile plastic culture plates that were 3.9 inches in diameter for UVC Top treatment groups or on 3 inch diameter quartz glass dishes (Technical Glass Products, Painesville Township, OH), for UVC bottom treatment groups. UVC light can penetrate quartz glass, and therefore would not block UVC light from the UVC bottom treatment groups. Both the PTC and SER shelves were disinfected with 10% bleach with a contact time of 15 minutes between each UVC exposure time.

Positive control groups were allowed to dry for approximately 90 minutes and then held at their respective treatment times without UVC exposure (5 minutes or 120 minutes) before virus recovery.

Virus Recovery

When the exposure time was completed, the inoculated coupon surfaces were eluted with 3 ml of PBS using a 2 ml plastic transfer pipette and allowed to collect into another sterile 3.9 in diameter cell culture plate. Once the entire surface of the coupon was covered with PBS the surface was re-eluted with the same 3 ml of PBS, 10 more times. During the elution step, the surface of each coupon was scratched with transfer pipette to re-suspend any remaining virus and cells onto the surface. The 3 ml mixture of PBS and virus +/- feces were collected into 5 ml snap cap tubes and placed immediately on ice and then stored in a -80°C freezer until virus titration was performed.

The collected samples were centrifuged at 4,200 x g for 10 min at 4°C, and the supernatant was filtered (0.22 µM). Virus titration was performed in 96-well plates of H1299 cells. Briefly, the filtered supernatant was 10-fold serially diluted and inoculated into semi-confluent of H1299 cells in 96-well plates, with 5 replicates for each dilution. This was incubated at 37°C for 1 hour and the supernatant was discarded. One hundred microliters (µl) of fresh medium was added per well. After 4 days of incubation at 37°C with 5% CO₂, CPE development was recorded. The supernatants were transferred to new 96-well plates and saved. The cell plates were rinsed once with PBS and the plates fixed with 80% cold acetone for at least 10 min and dried. Fifty to 100 µl of SVA-specific fluorescent antibody conjugate was added to each well and allowed to incubate at 37°C for about 1 hour. The conjugate was decanted and plates washed with PBS 3 times, for about 5 min per time. The plates were then read under fluorescence microscope. The virus titers were calculated according to the Reed and Muench method and expressed as the 50% tissue culture infective dose per ml (TCID₅₀/ml). Virus titer reduction caused by UVC is defined as the difference between the UVC treated groups and their relative positive control's log virus titer. A reduction of >4 log virus titer (reduction of 99.99%) was considered efficacious.

Statistical Analysis

All data was analyzed after logarithm transformation with base 10; this transformation is commonly seen in serial dilutions. A generalized linear model (GLM) is used to study the effects on log virus titer from virus type, treatment, location, and material. The variable surface (top vs bottom) is excluded from the analysis since GLM reveals a non-significant difference between top and bottom in the first place. Simple effect comparisons between the UVC treated groups and their relative positive controls were implemented to find out where the significant difference in treatment occurs. The analysis was conducted with SAS 9.4 (SAS Institute, Cary, North Carolina). A significant difference is concluded when P value < 0.05.

Results

There was no significant difference ($P > 0.77$) in virus log titer between the top and bottom inoculated surfaces of all UVC treated groups. All UVC treatment group's log virus titer were compared to their relative positive control. Almost all UVC treated groups virus log titers were significantly different than their relative positive controls ($P < 0.05$) except for UVC treated cloth with virus and PBS in the PTC, UVC treated plastic with virus and feces in the PTC and UVC treated cloth with virus and PBS in the SER. Plastic groups with virus and PBS in both the SER (T value: -25.80, lsmeans: -7.38, SE: 0.29, $P < 0.0001$) and PTC (T value: -25.36, lsmeans: -7.25, SE: 0.29, $P < 0.0001$) had the most significant difference in virus log titer between UVC treated groups and its positive control group, followed by cardboard groups with virus and PBS between

UVC treated groups and its positive control groups in the SER (T value -7.72, lsmeans -2.21, SE 0.29, $P < 0.0001$). UVC treated cloth and cardboard in the PTC had higher virus log titers than their relative positive controls (T value 2.33, lsmeans 0.67, SE 0.29, $P 0.02$). Figures 4 and 5 show the UVC treated group's lsmean virus log titer in comparison with their relative positive controls.

Discussion

Although SVA is not a reportable disease it still warrants a FAD investigation due to the requirement to report vesicular lesions and the inability to distinguish it from FMD and other vesicular FADs. More research is needed to explore ways to mitigate SVA outbreaks in swine herds and prevent disease. It is unknown how SVA is transmitted, but it can be speculated that it can be transmitted via fomites, similarly to FMDV. UVC is a technology that is being utilized more in the swine industry to decontaminate items being brought onto swine farms. This study evaluated UVC as a method to deactivate SVA on common surfaces (cardboard, cloth and plastic) commonly brought into swine farms under different challenged conditions.

There was no significant differences in log virus titer between any UVC treated bottom and top inoculated surfaces. This suggests that the metal wire shelving used in the PTC and SER in this study did not block a significant amount of surface area on the contaminated coupon during UVC exposure.

Research has been done on several viruses and bacteria to find a UVC lethal dose. The UVC lethal dose is unknown for SVA, however, there is a dose found for poliovirus 1 LSc2ab, which is 41 mJ/cm² for a 6 log reduction.⁵ Poliovirus 1 is in the same family *Picornaviridae*, as SVA and it can be speculated that SVA may have a similar UVC lethal dose. The maximum intensity recorded in pre-study work for the commercialized PTC was approximately 0.80 mW/cm², with an average intensity of 0.64 mW/cm². At a 300 second exposure time and average intensity, the dose well exceeds the required 41 mJ/cm² dose needed to inactivate poliovirus 1. These measurements were used to calculate the exposure time for the SER, based on the equation: $Dose (mJ/cm^2) = Intensity (mW/cm^2) * Time (s)$, and the inverse square law. Because the intensity at a 5 minute exposure time at approximately 20 in distance was known for the PTC, an approximate exposure time for the SER could be calculated. The intensity of the ESP D-Lux units were unknown before the study, and therefore this was the best approximation of exposure time at a given intensity to accomplish a dose greater than 41 mJ/cm². There was some variation in the intensity recordings taken during the study, particularly for the PTC. This could be attributable to a couple different reasons. The UVC meter was placed approximately in the same position each time the coupons were exposed to UVC in the PTC, however, it is possible that because the chamber is a smaller dimension than the SER and intensities were taken more frequently, a slight variation in angle or placement of the UVC meter may have attributed to this variation. Even though there was variation in the intensity recordings, each intensity reading still reached a dose well above 41 mJ/cm².

UVC disinfection effectively reduced the SVA virus log titer on the plastic UVC treated groups with PBS. The plastic UVC treated groups with PBS had over a 7 virus log titer reduction. Although a majority of the UVC treated groups in the SER and PTC were significantly lower and/or different from their relative positive controls, from a clinical standpoint, UVC disinfection

did not effectively reduce the SVA virus log titer on the cardboard and cloth surfaces with PBS or with feces and on plastic with feces. There have been several studies done suggesting that rough, irregular surfaces can shield viruses and bacteria from UVC light and decrease its efficacy.⁶ One study looked at UVC disinfection on plastic agar plates versus beef steak and found that UVC was more effective on smooth, plastic agar plates than the irregular surface of beef steak.⁷ Another study demonstrated that UVC disinfection was more effective at reducing bacterial counts on the skin of smooth surfaced fish than rough surfaced fish.⁸ More recently, UVC light was found to be effective at disinfecting contaminated metal surfaces but was not effective on cloth surfaces.⁹ Surface topography may be important when considering UVC disinfection due to the shielding effect of permeable and/or irregular surfaces such as cardboard and cloth. Tiny pores or crevices may effectively shield virus from UVC exposure. UVC cannot penetrate many materials, and can only sterilize surfaces of materials.¹⁰ Therefore, organic material such as feces, may also shield or block the virus from UVC light, decreasing its efficacy as a disinfectant in the presence of organic material.

Some of the UVC treated cardboard and cloth with virus and PBS and virus and feces had a higher virus titer recovered than their relative positive controls as seen in Figures 3 and 4. This could have been due to variation in the virus recovery technique for the different treatment groups. Virus could have soaked into the permeable, porous cloth and cardboard material and not have been fully recovered. Another explanation could be the day that the positive controls were performed. The experimental study took place over 3 days and to avoid contamination of the UVC treated groups during the study, the positive controls were inoculated and recovered on day 3 of the study, after the UVC treated groups, and the virus titer could have been slight decreased from its beginning titer of $3 \times 10^{7.5}$.

X. Tables and Figures:

Table 1: Details the three different surface materials evaluated under UVC disinfection.

Material	Description	Production Setting Represented
Cardboard	Permeable vaccine shipping boxes	Vaccine boxes and shipping supplies entered through supply entry room.
Plastic	Non-permeable, 1.5 mil polypropylene plastic bags	Supplies and lunches entered through UVC pass-through box and supply entry room.
Cloth	Permeable, cloth lunch bag	Lunch bags entered through UVC pass-through box.

Figure 1: The commercially available Bioshift® Pass Through Germicidal Chamber that was used in the study.



Figure 2: *A.*) The outside of the 8 by 8 ft supply entry room (SER) built for the study. *B.*) Inside the SER. A total of four ESP D-lux units were installed in the room, one centrally located at the top and bottom of each side wall of the SER.

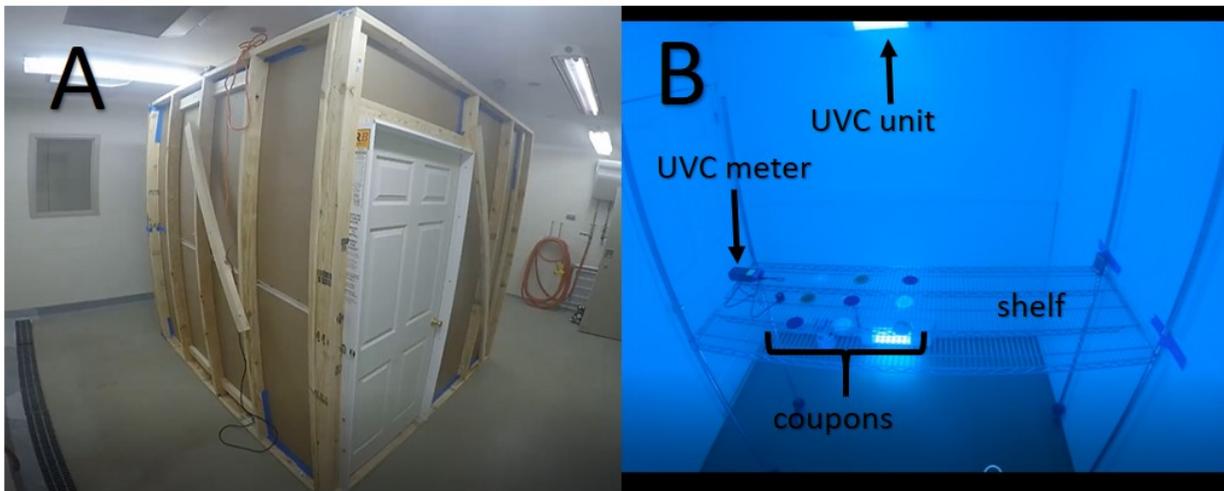
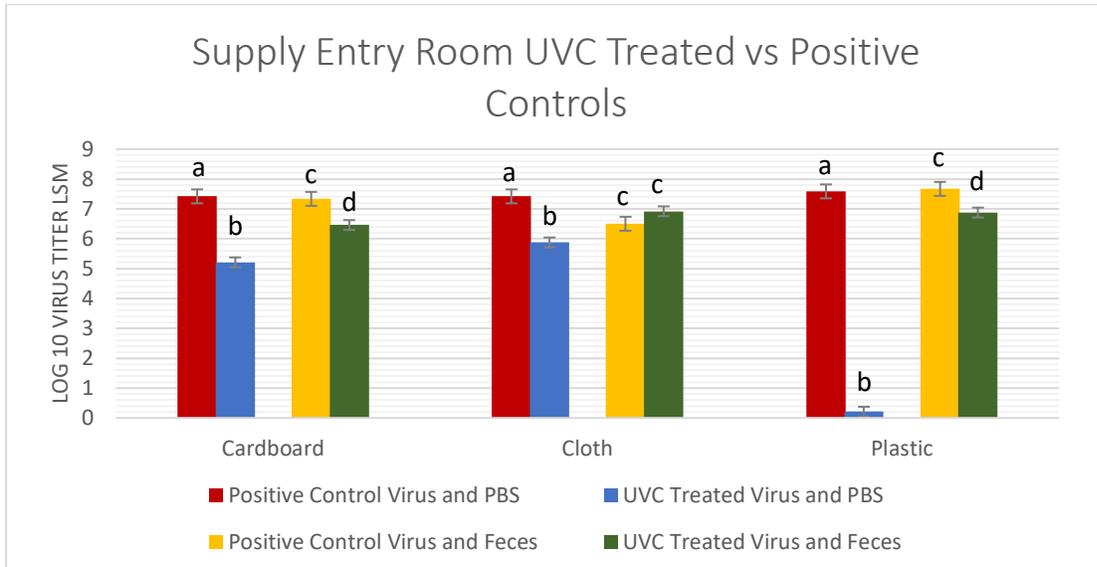
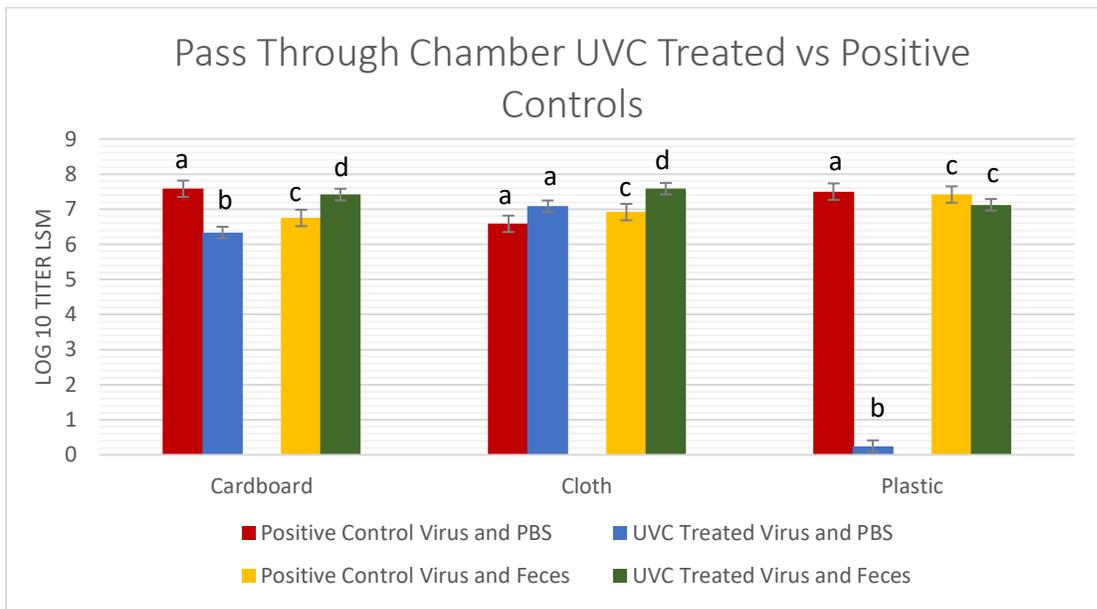


Figure 3: Comparison of the lsmeans virus log titers of the UVC treatment groups to their relative positive controls in the SER. Results shown are lsmeans of the three replicates per UVC treatment group and positive controls.



*abcd signifies if the lsmean virus log titer difference was significant compared to its relative positive control.

Figure 4: Comparison of the lsmeans virus log titer of the UVC treatment groups to their relative positive controls in the PTC. Results shown are lsmeans of the three replicates per UVC treatment group and positive controls.



*abcd signifies if the lsmear virus log titer difference was significant compared to its relative positive control.

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