RESEARCH REPORT

SWINE HEALTH

Title: Inactivation of African Swine Fever Virus on Stainless Steel and Concrete with Commercial Disinfectants and Organic Acids – **NPB #19-221**

Investigator: Lindsay Gabbert, MPS

Study Director: John Neilan, Ph.D.

Support scientists: Mariceny Zurita; Lauren Martignette, M.S.; Jose Barrera, DVM, Ph.D.

Institution: Department of Homeland Security Science and Technology Directorate, Office of National Labs, Targeted Advanced Development Group, Plum Island Animal Disease Center

Date Summary Submitted: February 14, 2022

Industry Summary:

The primary purpose of this research study was to determine if liquid microbicides commonly used in swine production facilities demonstrated virucidal efficacy against African swine fever virus (ASFV). In collaboration with National Pork Board (NPB) stakeholders, a list of common disinfectants was identified, and specific commercial off-the-shelf products were prioritized for testing. Commercially manufactured disinfectants evaluated in this study represented a wide range of chemically active ingredients and included Virkon™ S (potassium peroxymonosulfate), Virocid® (quaternary ammonium/ glutaraldehyde), Synergize® (quaternary ammonium/glutaraldehyde), Tek-Trol® (phenol), and Intervention® (hydrogen peroxide). Additionally, reagent-grade solutions of both citric and acetic acid (organic acids) were tested.

Using a standardized quantitative carrier test method published by the International Organization for Economic Cooperation and Development (OECD, 2013), the reduction in infectious ASFV was determined after exposure to each test chemical and acid solution at the concentration and contact time specified on the product labels for use against viruses of veterinary importance in farm

settings. Efficacy tests were conducted on non-porous stainless-steel discs and porous unpainted concrete test coupons using methods published by Gabbert, et al. (2020).

The U.S. Environmental Protection Agency (EPA) Antimicrobials Division requires demonstration of a minimum 3 $\log_{10}$ (99.9%) reduction in infectious viral titer to designate a disinfectant as efficacious for the purposes of product registration. Using this cutoff as our standard, and under the conditions tested, we determined that on non-porous stainless steel, Virkon™ S (1%), Virocid® (1:256), acetic acid (3%), and citric acid (3%) solutions met the minimum 3 $\log_{10}$ performance standard when ASFV was dried in a standardized soil load. Similar tests conducted on porous concrete demonstrated that Virocid® and Virkon™ S were capable of inactivating >3 $\log_{10}$ ASFV on that surface. These results suggest that some chemical disinfectants may require longer contact times or higher concentrations when used for the purpose of ASFV inactivation on porous concrete, and that acid solutions, while effective on non-porous stainless steel, have reduced efficacy when applied to concrete.

Of the 7 disinfectants evaluated in this study, only Virkon™ S and citric acid are registered by the EPA for use against ASFV and were included as internal benchmarks to validate the test method. Thus, completion of these efficacy tests resulted in the identification of two additional liquid disinfectants (Virocid® and acetic acid) which demonstrated virucidal efficacy by meeting the minimum 3 $\log_{10}$ reduction for ASFV inactivation.

Post-disinfection, sample eluates were analyzed by real-time polymerase chain reaction (RT-PCR) to determine whether exposure to the test chemicals resulted in an appreciable reduction in the ASFV DNA signal. In general, minimal changes in cycle threshold (Ct) values were observed after the 10-minute contact time. RT-PCR can detect very small segments of viral DNA, so while degradation of the ASFV nucleic acid genome may occur after contact with chemical disinfectants, it is insufficient to destroy DNA beyond the assay limit of detection.

In this report we provide efficacy data obtained via standardized test methods to allow industry stakeholders to proactively choose disinfectants that are effective against ASFV. Chemicals with similar active ingredients may vary in overall ability to inactivate virus, thus generalizations should not be made among products perceived to be similar in chemical formulation.

Contact: Lindsay.Gabbert@ST.DHS.Gov

Key Findings:

- Virocid® (1:256), Virkon™ S (1%), acetic acid (3%), and citric acid (3%) were the most effective chemical disinfectants when applied to non-porous stainless steel—with all test chemicals inactivating > 4 $\log_{10}$ of infectious ASFV.

- Virocid® (1:256) and Virkon™ S (1%) were the only test chemicals that met the EPA required minimum 3 $\log_{10}$ (99.9%) reduction in ASFV when applied to unsealed porous concrete.

- The two commercial products containing quaternary ammonium compounds (Virocid® and Synergize®) demonstrated differences in efficacy of 1.9 to 2.0 $\log_{10}$ (79- to 100-fold) on stainless steel and concrete, suggesting that product formulation may play an important role when comparing disinfectants with similar active ingredients.

- Disinfection of ASFV with the 7 test chemicals did not degrade viral DNA below detection limits of the highly sensitive RT-PCR test. Therefore, sampling of contaminated premises post-cleaning and disinfection may yield positive RT-PCR results that do not indicate the presence of infectious ASFV.

Keywords: Disinfection, African swine fever virus, cleaning, decontamination, transboundary animal disease

Scientific Abstract:

An outbreak of ASFV in the U.S. would greatly affect the continuity of domestic pork production and restrict export of U.S. pork and pork-derived products. Strict biosecurity practices currently serve as the most effective measure for ASFV control. Identification of additional chemical disinfectants that effectively inactivate ASFV will provide actionable data for the development of robust cleaning and disinfection (C&D) procedures on non-porous and porous surfaces in the response and recovery phases of an ASFV outbreak. Presently, there are seven disinfectants registered by the U.S. EPA under The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 3 for use against ASFV (APHIS, 2021). Three additional chemicals are approved under FIFRA Section 18 for emergency use when no registered products are available. Importantly, many of these registered disinfectants are not routinely used by pork producers and may not be readily available in large quantities during an ASFV outbreak if disruptions in product supply chains occur. The purpose of this study was to determine whether products commonly in use at swine

production facilities were capable of inactivating ASFV on porous and non-porous surfaces.

All tests were conducted according to protocols published in the *OECD Quantitative Method for Evaluating Virucidal Activity of Microbicides used on Hard Non-Porous Surfaces* (OECD, 2013). High-titer stocks of the Vero cell-adapted ASFV strain BA71V were combined with a standard soil load comprised of bovine mucin, yeast extract, and bovine serum albumin (BSA). Viral inocula were dried on either stainless steel or concrete coupons (n=7/test) and exposed to each disinfectant for10-minutes. Test chemicals were neutralized, and the sample eluates were titrated on Vero cells to determine the 50% tissue culture infectious dose ($TCID_{50}$) based on viral cytopathic effects (CPE) after 8 days. Results are reported as the log_{10} reduction in infectious ASFV when compared with untreated control samples (n=3/test). Each test was conducted on two days to increase statistical confidence.

Test results for stainless steel and (concrete) demonstrated reductions in viable ASFV (log_{10} $TCID_{50}$/mL) of 5.5 (0.4) for acetic acid (3%); 4.8 (4.1) for Virocid® (1:256); 4.7 (3.2) for Virkon™ S (1%); 4.4 (2.1) for citric acid (3%); 2.9 (2.1) for Synergize® (1:256); 2.7 (1.8) for Tek-Trol®; and 2.0 (1.3) for Intervention®.

The data obtained in this study provide valuable information for pork producers concerned with choosing effective liquid disinfectants for non-porous and porous surface cleaning and virus elimination on ASFV-infected premises. Additionally, two chemical disinfectants that were not previously registered under FIFRA demonstrated the ability to inactivate > 4 log_{10} of infectious ASFV on non-porous (Virocid ® and acetic acid) and porous (Virocid ®) surfaces.

Introduction:

African Swine Fever (ASF) is considered endemic in many countries and has continued to spread rapidly throughout Western/Eastern/Southern Asia since 2018. As a result, its further emergence in ASF-free zones remains a danger. Most recently, in July and September 2021, ASFV was officially detected in the Dominican Republic and Haiti, respectively. This is the first detection of ASFV in the Americas in approximately 40 years, and the geographical closeness to the U.S. mainland and territories heightens the risk of further introduction to U.S. agriculture.

ASFV infects domestic and wild pigs, with up to a100% acute mortality rate following infection with some ASFV genotypes/strains in domestic pigs. Large outbreaks in China beginning in August 2018 to the present have reduced the number of production hogs by at least 40%. Currently there are no effective treatments or licensed vaccines available. ASFV spreads through multiple routes of transmission and thus remains a highly transmissible OIE trade-

restricting transboundary animal disease. ASFV is unable to infect humans and presents no public health risk.

ASFV is a large, enveloped, nucleocytoplasmic DNA virus and the sole member of the *Asfarviridae* family. The virus particle is stable over a wide pH range (3-12) and has been shown to survive for long periods of time in the environment and in infected swine tissues, especially at low temperatures and in moist conditions. Thus, environmental contamination of production sites, fomites, feed, and transportation networks may significantly contribute to farm-to-farm disease transmission. In the event of an ASFV outbreak, significantly increased use of disinfectant products is expected to occur across all sectors of pork production, including but not limited to, farms, cleaning and disinfection stations, slaughterhouse and rendering facilities, and transportation networks for live-haul, feed, fuel, and service vehicles.

Due to the absence of currently licensed ASF vaccines, the principal approach to ASF control is based on stringent biosecurity measures, mainly the implementation of sanitary procedures and immediate disease detection. Effective disinfection of environmental surfaces contaminated with ASFV is crucial to respond and recover from any ASF outbreak in the U.S. domestic pig herd. Use of validated disinfection protocols following an ASF outbreak will ensure continuity of business operations.

In the U.S., commercial manufacturers seeking product registration and a label claim for use of disinfectants against ASFV must furnish data demonstrating product effectiveness to the EPA for review. Currently accepted EPA efficacy testing standards are outlined in the *Product Performance Test Guideline, OCSPP 810.2200, Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing* (EPA, 2018). For virucidal testing specifically, the currently accepted test method, published by the American Society for Testing and Materials (ASTM), is ASTM E1053 (ASTM, 2020). In general, quantitative carrier tests utilizing the specific virus of interest, dried on test coupons in the presence of a soil load, are preferred over liquid-based suspension tests for agricultural pathogens, as they present a more challenging test for the disinfectant.

Efficacy testing in the U.S. is complicated by the fact that ASFV is designated as a select agent virus by the Federal Select Agent Program, and thus can only be manipulated in approved Biosafety Level 3 (BSL3) laboratories that possess entity registration for storage and handling of ASFV. Due to this requirement, few qualified laboratories exist with the capacity to conduct disinfectant efficacy tests meeting EPA regulatory standard requirements. The ASTM E1053 method presents challenges for testing of ASFV due to the large volume of virus required, ASFV's tendency to desiccate after drying, and the large volume of disinfectant (2 mL) used which often results in cytotoxicity to mammalian host cell line(s) utilized for virus isolation.

In this report we present virucidal efficacy data for 7 test chemicals using the disc-based OECD quantitative carrier test method. High-titer ASFV stocks were produced to overcome loss of virus due to drying—allowing for sufficient recovery titers to demonstrate a minimum 3 $\log_{10}$ (99.9%) reduction in infectious virus after disinfection. In addition, this specific test method minimizes cytotoxicity to host cell lines through a relatively higher neutralizer to disinfectant ratio. Lastly, this method may be modified for use with other select agent transboundary animal disease viruses of agricultural importance to identify effective disinfectants.

Objectives:

Evaluate the ability of commercially available chemical disinfectants and acid solutions relevant to the U.S. pork production industry for their ability to inactivate ASFV on both non-porous and porous surfaces. Specifically:

(a) Determine the virucidal efficacy of 7 NPB-selected test chemicals using a standardized quantitative carrier test on non-porous (stainless steel) and porous (unpainted concrete) surfaces per manufacturers' recommendations (i.e., chemical field-use concentration and contact time).

(b) Determine whether ASFV inactivation, as measured by virus isolation, correlates with a reduction in detectable viral DNA via RT-PCR in disinfected samples.

(c) Submit final technical reports for test chemicals that demonstrate a minimum 3 $\log_{10}$ reduction in ASFV titer to the U.S. EPA in support of registration, under FIFRA Section 3 or Section 18 Quarantine Exemption, so that products may be used in ASFV outbreak response by U.S. pork producers and emergency response agencies.

Materials & Methods:

ASFV Stock Production

Vero cells were cultured in complete growth media (cDMEM) consisting of Dulbecco's Modified Eagle Medium (DMEM) plus 10% fetal bovine serum (FBS), 1X antibiotic-antimycotic (A/A), 1 mM sodium pyruvate, and 1X non-essential amino acids. ASFV lab strain BA71V ("ASFV" or "test virus") was amplified by incubation on Vero cells for 5 days. Supernatants from infected cells were harvested 5 days post-infection and clarified by centrifugation. ASFV was concentrated from supernatants by the addition of 8% polyethylene glycol (PEG-8000) and 2.3% NaCl with continuous slow stirring at 4°C for 18 hours. PEG/NaCl-ASFV supernatant was centrifuged at 3,200 x g for 30 min at 8°C, and

the precipitate was reconstituted in DMEM-3% A/A to 1/100 of the original supernatant volume. The viral stocks were titrated to obtain the 50% Tissue Culture Infectious Dose ($TCID_{50}$/mL) values prior to quantitative carrier tests. Single-use aliquots of this virus stock were made to avoid freeze-thaw cycles and ensure repeatability among subsequent tests; viral stocks were frozen at -80°C.

Disinfectant Neutralization and Cytotoxicity Testing

Disinfectant neutralization and cytotoxicity tests were performed prior to efficacy testing to rule out any non-specific negative effects to the Vero cells and virus due to exposure to, or continued action of, the disinfectant after neutralization at the desired contact time. To determine whether the neutralizer impacted ASFV survival, viral inocula were diluted to 10^2-10^3 infectious virus particles per test. At intervals of 30 seconds ± 3 seconds, 10 µL of the virus inoculum was added to flat-bottomed Nalgene vials containing either 10 mL of phosphate buffered saline (PBS) (n=3) or 10 mL of the neutralizer (cDMEM + 2% FBS) (n=3).

To ensure that the test chemicals were effectively neutralized, six additional vials containing 50 µL (n=3) or 100 µL (n=3) of the test chemical received 10 mL neutralizer at 30-second intervals for a 10-second contact time prior to the addition of 10 µL of the virus. Viral suspensions were serially diluted ten-fold from 10^0 to 10^{-3} and plated in replicates of 6 wells/dilution on 48-well plates (CytoOne #CC7682-7548) containing Vero cells at ~80% confluency.

Neutralizer effectiveness was determined by comparison of virus titers from PBS controls and the vials containing the test chemical. A difference in titer of >0.5 log_{10} between the control group and the treatment containing the disinfectant was considered a failed neutralization. Previous tests with citric and acetic acids demonstrated cDMEM + 2% FBS to be an effective neutralizer (data not shown), and thus retesting was not performed for these chemicals.

Cytotoxicity testing consisted of mixing 50 µL of disinfectant with 10 mL of neutralizer and plating the 10^0 – 10^{-2} diluted suspensions on Vero cells in 48-well plates. Cell monolayers were observed for 8 days to determine if cell growth and survival were impacted.

Disinfectant Efficacy Testing

All experiments were conducted in accordance with the *OECD Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-porous Surfaces, Section D: Quantitative Method for Evaluating Virucidal Activity of Microbicides used on Hard Non-Porous Surfaces* (OECD, 2013).

Disinfectant solutions were prepared on the morning of each test day by dilution in OECD hard water (target hardness 375 ppm $CaCO_3$). Each commercial disinfectant was tested at the concentration recommended for virus inactivation

on the respective product labels (Table 1). All labels recommended a 10-minute contact time. Reagent-grade citric and acetic acids were tested at a concentration of 3% based on current guidelines established by USDA Animal and Plant Health Inspection Service (APHIS) for use of citric acid against Foot-and-mouth-disease virus (FMDV) and ASFV during transboundary animal disease outbreaks (USDA, 2019). All tests were conducted at room temperature.

Table 1. Preparation of Test Chemicals

Name	Final Concentration	Volume Disinfectant	Volume Hard Water	Final pH
Acetic Acid	3%	1.2 mL	38.80 mL	2.6
Citric Acid	3%	1.2 g (w/v)	38.80 mL	2.0
Virkon™ S	1%	0.4 g (w/v)	39.60 mL	2.4
Intervention®	1:64	625 µL	39.38 mL	2.3
Synergize®	1:256	156 µL	39.80 mL	6.9
Tek-Trol®	1:256	156 µL	39.80 mL	9.8
Virocid®	1:256	156 µL	39.80 mL	7.4

Note: Commercial disinfectants used in the study were procured from QC Supply (Schuyler, Nebraska); acetic and citric acid were purchased from MilliporeSigma.

Stainless steel coupons were prepared according to OECD standards, and concrete testing coupons were prepared as described previously by Gabbert, et al. (2020). Concrete coupons were carbonated for 1 week in a 5% CO_2 incubator, a process that lowers the surface and total concrete matrix pH to approximately 8.5-9 (Gabbert, *et al.* 2020), thus allowing for a more favorable porous surface from which to recover virus. All coupons were sterilized by autoclaving at 121ºC for 15 minutes prior to testing.

Sterile coupons were arranged in glass petri dishes and inoculated with 10 µL of the virus test suspension using a positive displacement pipette. Test suspensions were prepared to contain final concentrations of 0.35% tryptone, 0.25% bovine serum albumin, and 0.04% bovine mucin as the standard soil load. For each experiment (test day), 3 replicate coupons were included as positive controls, and 7 replicate coupons were used to test each chemical disinfectant. Viral suspensions were dried on coupons for 1 hour in a Class II biosafety cabinet before transfer to sterile flat-bottomed Nalgene plastic cups (1 coupon/cup). At intervals of 30 seconds, 50 µL (100 µL for concrete) of either the test disinfectant, or cDMEM for controls, was overlaid on the dried virus inoculum. After the 10-minute (± 3 second) contact time, 10 mL of cDMEM + 2% FBS (neutralizer) was added to each cup to stop the reaction. The coupons were vortexed for 30 seconds to recover any remaining viable virus in the media suspension.

Eluates from stainless steel coupons were serially diluted 10-fold by mixing 500 µL of inoculum with 4.5 mL of cDMEM and repeated until required dilutions

were achieved. Serially diluted eluates were titrated on 48-well plates containing monolayers of ~80% confluent Vero cells by adding 500 µL per dilution to each well in replicates of six. Test and control carrier plates were incubated at 37°C in 5% CO_2 for 8 days. Each well was visually observed for the presence of CPE and scored. The $TCID_{50}$/mL values were determined using the Reed and Muench method.

Viral DNA Detection Post-Disinfection

To assess whether exposure to the test chemicals degraded ASFV DNA, eluted sample supernatants were also analyzed via real-time polymerase chain reaction (RT-PCR) using primers and probes specific for amplification of the gene sequence encoding the major ASFV p72 capsid protein. Briefly, disinfection tests were conducted as described above, using three replicate stainless steel test coupons per disinfectant and a contact time of 10 minutes. Virus was eluted in a final volume of 10 mL. A 1 mL aliquot from each neutralized sample was stored at -80º C until analysis. Untreated ASFV coupons and media only samples were included as positive and negative controls.

Samples were analyzed using the diagnostic protocol kindly provided by the USDA APHIS National Veterinary Services Laboratory (Grau, F., *et al.*, 2015). Briefly, each 1.0 mL sample tube was thawed, vortexed, and pulse spun before testing. The MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher, Cat. No. 4462359) and the TaqMan™ Fast Virus 1-Step Master Mix (Thermo Fisher, Cat. No. 4444436) were used for manual extraction and RT-PCR detection. Extracted DNA samples were amplified in replicates of three. The probe 5' 6FAM- CgA TgC AAg CTT TAT -3' MGB/NFQ, along with forward primer 5'-CCT Cgg CgA gCg CTT TAT CAC-3' and reverse primer 5'-ggA AAC TCA TTC ACC AAA TCC TT-3', were used to detect ASFV DNA on an Applied Biosystems 7500 instrument. Cycling conditions consisted of denaturation at 95°C for 20 seconds (1 cycle), 45 cycles of amplification at 95°C for 10 seconds, and 60°C for 30 seconds. Threshold cycle (Ct) values of up to 40 were considered positive.

Results:

ASF Virus Stock Production

After precipitation with PEG, concentrated ASFV stock titers of approximately 9.1 $\log_{10}$ $TCID_{50}$/mL were obtained. Mixing of the virus stock with the 3-component soil load reduced the starting titer by 0.68%. Of this inocula, 10 µL was deposited on each test coupon. Preliminary tests demonstrated that >6.0 $\log_{10}$ $TCID_{50}$/mL of infectious ASFV was recovered after drying the virus inoculum (average of both control coupons [stainless steel and concrete]; data not shown). A minimum recovery of 5.3 $\log_{10}$ $TCID_{50}$/mL of virus was necessary to

calculate a final >3 log_{10} reduction as required by the U.S. EPA to demonstrate product efficacy (based on an assay limit of detection of 1.3 log_{10}).

Disinfectant Neutralization and Cytotoxicity Testing

As found previously for citric acid and acetic acid, four test chemicals were effectively neutralized with cDMEM + 2% FBS (Table 2). One disinfectant (Intervention®) required addition of bovine catalase (0.1%) to the neutralizing solution, as this enzyme is responsible for catalyzing the decomposition of hydrogen peroxide (the product's active ingredient). Additionally, the neutralizing conditions had a minimal effect on the titer of ASFV, ≤0.5 log_{10} reduction, when compared to treatment groups containing no test chemical (Table 2). Synergize® (1:256), Virkon™ S (1%), and Virocid® (1:256) were toxic to the Vero cell line at the 10^0 dilution (undiluted) when 100 µL was used. However, cytotoxicity was abolished by dilution of the media 1:10, allowing for adequate titer calculations.

Table 2. Neutralization Test Results

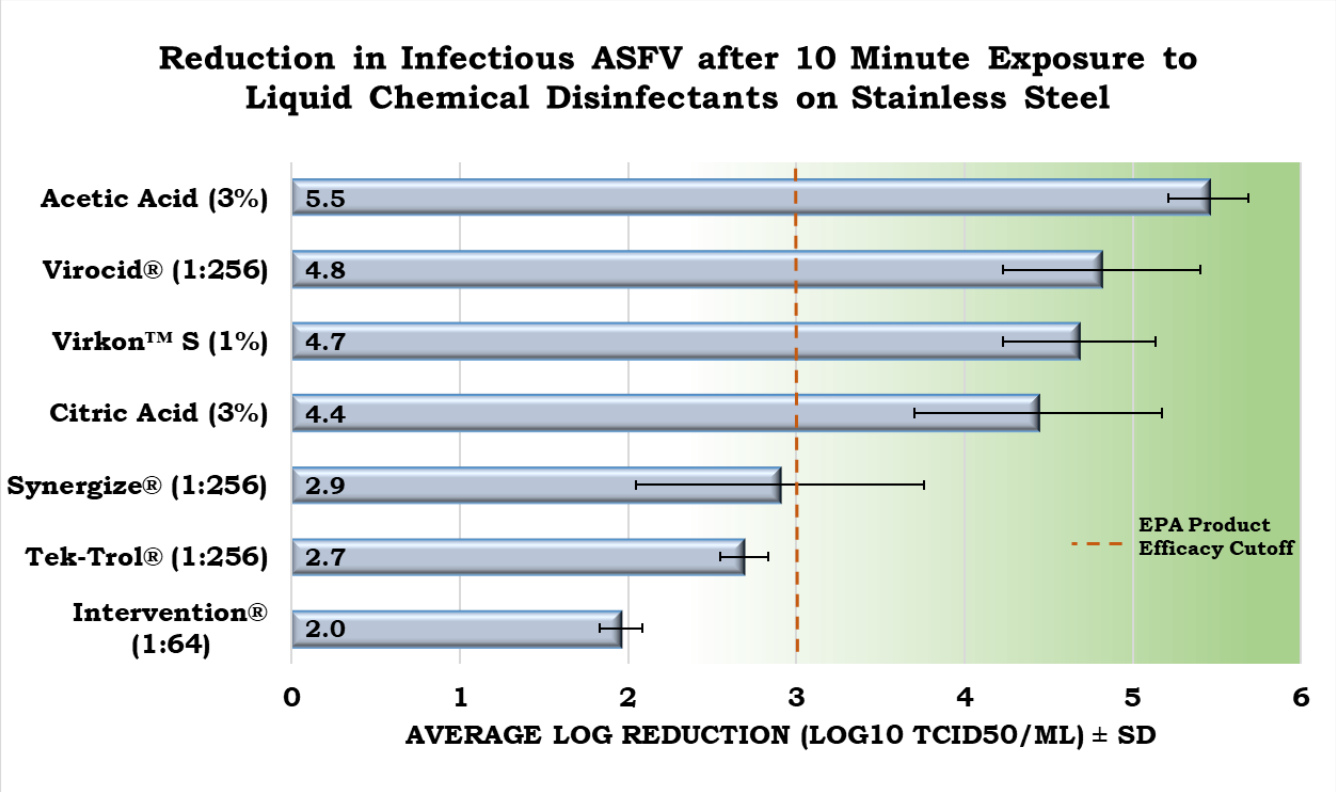
Commercial Disinfectant	Concentration Tested	Volume Tested (µL)	Neutralizer (10 mL)	Cytotoxicity (Yes/No)	Log_{10} Reduction over Controls
Synergize®	1:256	50	cDMEM + 2% FBS	No	0.00
		100	cDMEM + 2% FBS	Yes (10^0)	0.25
Tek-Trol®	1:256	50	cDMEM + 2% FBS	No	0.10
		100	cDMEM + 2% FBS	No	0.20
Intervention®	1:64	50	cDMEM + 2% FBS + 0.1% Bovine Catalase	No	0.30
		100	cDMEM + 2% FBS + 0.1% Bovine Catalase	No	0.30
Virkon™ S	1%	50	cDMEM + 2% FBS	No	0.22
		100	cDMEM + 2% FBS	Yes (10^0)	0.20
Virocid®	1:256	50	cDMEM + 2% FBS	No	0.18
		100	cDMEM + 2% FBS	Yes (10^0)	0.22

Disinfectant Efficacy Testing

Each disinfectant was tested against ASFV on two test days to evaluate experimental repeatability. Tests with an average virus control recovery titer of less than 5.3 $\log_{10}$ $TCID_{50}$/mL were excluded from analysis and re-tested. The average recovery of ASFV from untreated control coupons (n=3 from 8 different test days), was 6.3 $\log_{10}$ (± 0.6) for stainless steel, and 5.8 $\log_{10}$ (±0.9) for concrete (data not shown).

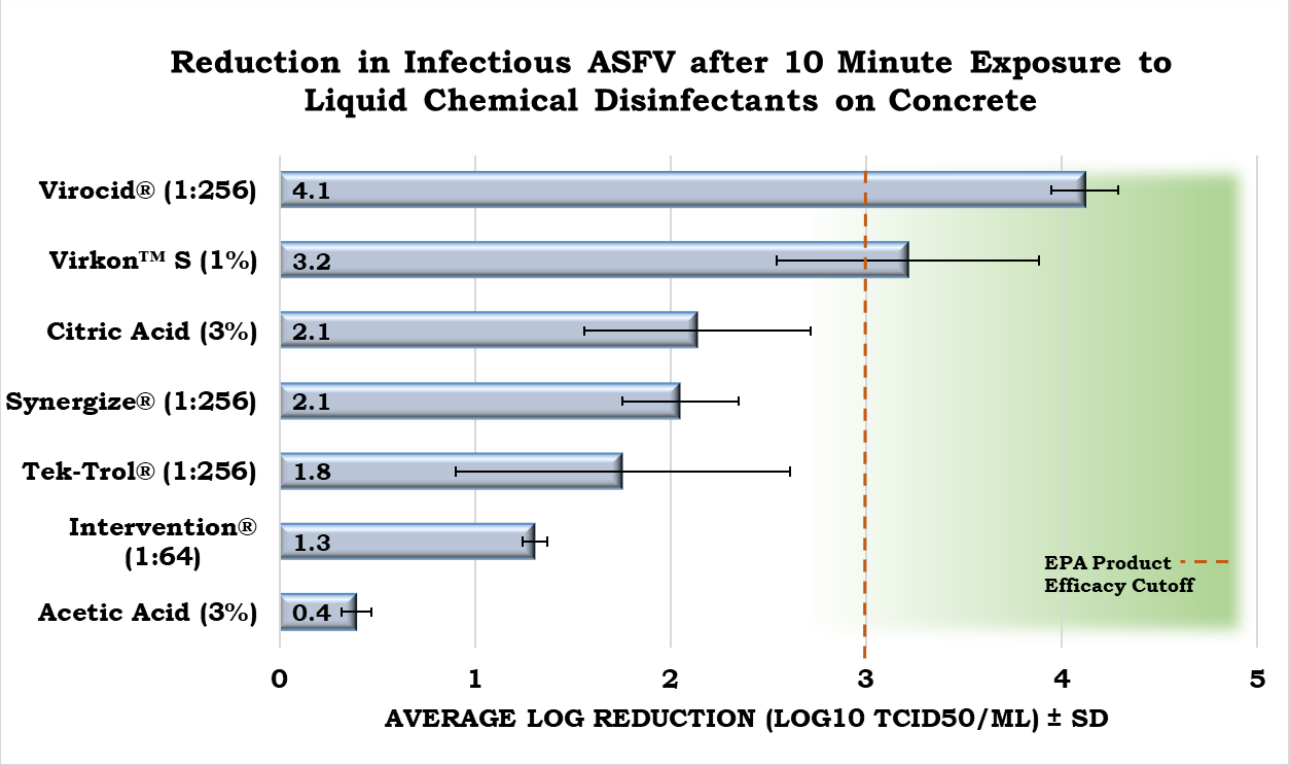
Virucidal efficacy results demonstrated that after a 10-minute contact time on stainless steel, 3% acetic acid, 3% citric acid, 1% Virkon™ S, and Virocid® (1:256), reduced the infectious titer of ASFV by greater than 3 $\log_{10}$ $TCID_{50}$/mL (>99.9%). Synergize®, Tek-Trol®, and Intervention® did not meet the efficacy requirements (Figure 1). However, it should be noted that Synergize® had an average log reduction of 2.3 $\log_{10}$ on test day 1, and 3.5 $\log_{10}$ on test day 2, in which case data from test 2 would meet regulatory standards.

Figure 1. Quantitative efficacy results for inactivation of ASFV on non-porous stainless steel following a 10-minute exposure to the test chemicals. Log_{10} reduction values are the average of two tests conducted on separate test days (n=14 replicates).



Inactivation of ASFV on concrete proved to be more difficult than stainless steel. Only Virocid® (1:264) and 1% Virkon™ S reduced infectious virus by ≥3 log_{10} $TCID_{50}$/mL (Figure 2). In general, the relative ranking of the disinfectants on concrete and stainless steel was similar with the exception of acetic acid, which was least effective on concrete.

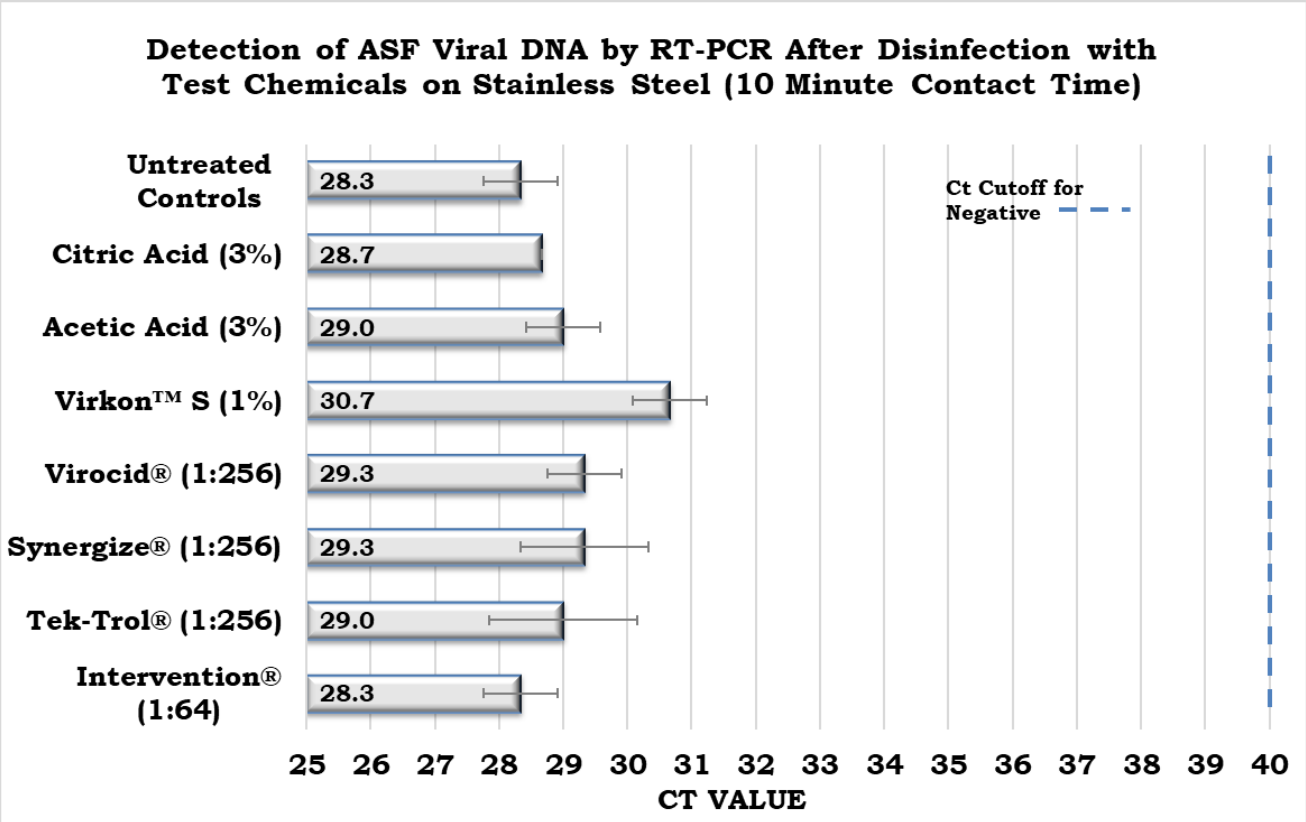
Figure 2. Quantitative efficacy results for inactivation of ASFV on concrete following a 10-minute exposure to the test chemicals. Log_{10} reduction values are the average of two tests conducted on separate test days (n=14 replicates).



ASFV Viral DNA Detection

Eluates from untreated control coupons (stainless steel) had an average RT-PCR Ct value of 28.3. After disinfection, no chemical treatment significantly reduced the ASFV viral DNA signal, as demonstrated by little to no change in Ct values of test chemical eluates (Figure 3). Treatment of dried ASFV with Virkon™ S resulted in the largest difference compared with controls, but the average change in Ct value of 2.4 was negligible. A sample negative for ASFV p72 DNA would have a Ct value of 40 or higher. These results demonstrate that despite inactivation of infectious ASFV, as established by virus isolation, the chemicals tested did not eliminate the presence of detectable ASFV DNA.

Figure 3. RT-PCR results demonstrating average Ct values for detection of ASFV p72 DNA in eluates of disinfected coupons (n=3 per disinfectant and untreated control)



Discussion:

Quantitative virucidal efficacy test results demonstrated that four of the seven liquid disinfectants effectively inactivated ASFV dried on stainless steel in the presence of a standardized soil load after a 10-minute contact time. Of the four effective chemicals, Virkon™ S is registered for use against ASFV by EPA under the FIFRA Section 3, and 3% citric acid is registered under FIFRA Section 18 Emergency Quarantine Exemption. Thus, our results are consistent with previous data demonstrating product efficacy for these two chemical disinfectants.

To the best of our knowledge, virucidal efficacy data for Virocid® against ASFV has not been publicly reported, but under the conditions tested, this commercial disinfectant met the requirements for product efficacy per guidelines established by EPA. Acetic acid is available commercially as a chemical reagent but is not marketed as a commercial disinfectant. On non-porous stainless steel, it demonstrated the greatest reduction in infectious ASFV of the seven tested chemicals, and thus would satisfy the requirements for Section 18 Quarantine Exemption for use in the event of an ASFV outbreak, particularly as it is considered "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration and thus acceptable for use on food contact surfaces (FDA, 2021).

Each chemical disinfectant in this study was tested using the OECD Quantitative Test Method because it requires a relatively higher threshold of activity for the test chemicals, similar to what may be encountered on swine production sites. The product concentrations and contact times were chosen based on the EPA-published label instructions for use against other viral pathogens in farm settings. It is possible that under different parameters, such as use of a suspension test (EN14675) or another quantitative carrier test (ASTM 1053E) method, results may differ (ASTM, 2020; European Standards, 2015). Additionally, higher product concentrations or longer contact times may increase efficacy against ASFV on the surface types tested, resulting in more products meeting the required minimum 3 $\log_{10}$ (99.9%) reduction in infectious ASFV.

As project deliverables, final technical data reports were provided to the EPA and APHIS-Veterinary Services in support of obtaining Section 3 registration for use of Virocid®, and a Section 18 Quarantine Exemption for use of acetic acid, against ASFV.

Funding for this project was provided in part by the National Pork Checkoff as part of a funded Cooperative Research and Development Agreement (CRADA) (20-PIADC-001) with the Department of Homeland Security Science and Technology Directorate.

References

ASTM (2020). ASTM E1053-20: Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces. Available from https://www.astm.org/e1053-20.html

EPA (2018). Product Performance Test Guideline, OCSPP 810.2200, Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, [EPA 712-C-17-004]. Available from https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0036

European Standards (2015). CSN EN 14675 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area. Available from https://www.en-standard.eu/csn-en-14675-chemical-disinfectants-and-antiseptics-quantitative-suspension-test-for-the-evaluation-of-virucidal-activity-of-chemical-disinfectants-and-antiseptics-used-in-the-veterinary-area-test-method-and-requirements-phase-2-step-1/

FDA (2021). Food and Drugs, 21 C.F.R. § 184.005 (1982). Available from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1005

Gabbert LR, Neilan JG, Rasmussen M. (2020). Recovery and chemical disinfection of foot-and-mouth disease and African swine fever viruses from porous concrete surfaces. J Appl Microbiol. 2020 Nov;129(5):1092-1101. doi: 10.1111/jam.14694.

Grau FR, Schroeder ME, Mulhern EL, McIntosh MT, Bounpheng MA. (2015). Detection of African swine fever, classical swine fever, and foot-and-mouth disease viruses in swine oral fluids by multiplex reverse transcription real-time polymerase chain reaction. J Vet Diagnostic Invest 27: 140–149. https://doi.org/10.1177/1040638715574768.

LJ Reed and H Muench. 1938. A simple method of estimating fifty percent endpoinrts. Am. J. Epidemiology. 27 (3):493-497, https://doi.org/10.1093/oxfordjournals.a-je.a11840.

OECD Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces. 2013. Available from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)11&doclanguage=en

USDA APHIS (2021). Disinfectants Approved for use Against African Swine Fever Virus in Farm Settings. Available from

https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-virus-disinfectants.pdf

USDA APHIS (2019). FIFRA Section 18 Emergency Exemption Label: Citric Acid. Available from https://www.aphis.usda.gov/animal_health/emergency_management/downloads/19citricacid-exemption-label.pdf