SMART CARD DECONTAMINATION IN A HIGH-CONTAINMENT LABORATORY

Lindsay R. Gabbert, Justin D. Smith, John G. Neilan, Geoffrey S. Ferman, and Max V. Rasmussen

Validated procedures for decontamination of laboratory surfaces and equipment are essential to biosafety and biorisk programs at high-containment laboratories. Each high-containment laboratory contains a unique combination of surfaces, procedures, and biological agents that require decontamination methods tailored to specific facility practices. The Plum Island Animal Disease Center (PIADC) is a high-containment laboratory operating multiple biosafety level (BSL)-3, ABSL-3, and BSL-3 Ag spaces. The PIADC facility requires the use of federally issued smart cards, called personal identity verification (PIV) cards, to access information technology (IT) networks both outside and within the high-containment laboratory. Because PIV cards may require transit from the BSL-3 to office spaces, a validated procedure for disinfecting PIV card surfaces prior to removal from the laboratory is critical to ensure biosafety and biosecurity. Two high-risk select agents used in the PIADC high-containment laboratory are foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV). We evaluated disinfection of PIV cards intentionally spotted with FMDV and SVDV using a modified quantitative carrier test and the liquid chemical disinfectant Virkon® S. Our experimental design modeled a worst-case scenario of PIV card contamination and disinfection by combining high concentrations of virus dried with an organic soil load and use of aged Virkon® S prepared in hard water. Results showed that FMDV and SVDV dried on PIV card surfaces were completely inactivated after immersion for 30 and 60 seconds, respectively, in a 5-day-old solution of 1% Virkon® S. Therefore, this study provided internal validation of PIADC biosafety protocols by demonstrating the efficacy of Virkon® S to inactivate viruses on contaminated smart cards at short contact times.

Keywords: Smart card, Personal identity verification card, Foot-and-mouth disease virus, Swine vesicular disease virus, Decontamination, Disinfection, High-containment laboratory, Select agent, Virkon® S

Lindsay R. Gabbert, MPS, is a Life Scientist at Leidos, and Justin D. Smith, MS, was an ORISE Fellow and a Life Scientist at Leidos; John G. Neilan, PhD, is the Director; and Max V. Rasmussen, PhD, is a Microbiologist; all at the US Department of Homeland Security Science Group, Plum Island Animal Disease Center, Greenport, NY. Geoffrey S. Ferman, MS, is Biological Safety Officer, US Department of Homeland Security, Plum Island Animal Disease Center, Greenport, NY.

© Lindsay R. Gabbert *et al.*, 2018; Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative Commons Attribution Noncommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any non-commercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

THE PLUM ISLAND ANIMAL DISEASE CENTER (PIADC) is the only laboratory in the United States authorized to work with many of the high-consequence agricultural pathogens that are not endemic to the United States. The list of biological agents used at the PIADC includes the federally regulated select agents of foot-and-mouth disease virus (FMDV), a US Department of Agriculture Tier-1 select agent, and swine vesicular disease virus (SVDV). As US federal regulations and guidance for working with these select agents evolve, the requirements for maintaining biosafety and biosecurity at high-containment laboratories must be continually reviewed and revised. Recent amendments to the Agricultural Bioterrorism Protection Act of 2002, Select Agents and Toxins Regulations (March 2017), highlight the need for high-containment laboratories working with select agents to develop site- and process-specific “validated inactivation procedures” based on pathogen biorisk level in materials after removal from containment.¹ Furthermore, Federal Select Agent Program policy guidelines indicate that inactivation procedures must be validated based on “in-house” testing protocols and supported by efficacy data produced by the specific high-containment laboratory.²

Biohazard risk assessments consider pathogenicity, transmissibility, and environmental persistence of pathogens, along with the anticipated risks of potential contamination and release scenarios. Pathogens may also be ranked for resistance to physical and chemical disinfection to indicate their inherent stability and ease of inactivation.^{3,4} For chemical inactivation, viral pathogens generally fall into 1 of 2 categories: the more labile large lipid-enveloped viruses, and the small nonenveloped viruses, which are more resistant to disinfectants and more environmentally persistent.³ FMDV and SVDV are 2 high-risk pathogens used at PIADC that fall into the latter category of small nonenveloped viruses resistant to inactivation by drying^{5,6} and require validated procedures for chemical disinfection.

Homeland Security Presidential Directive 12 (HSPD-12): Policy for a Common Identification Standard for Federal Employees and Contractors (July 2015),⁷ as well as other policies,⁸ require all federal employees and contractors to use a common identification standard for secure access to government facilities and information systems. To comply with HSPD-12, the PIADC uses personal identity verification (PIV) cards, a type of US federal smart card, as a form of 2-factor authentication to access all facility computers. For a minority of PIADC users, this requires transit of PIV cards into the high-containment laboratory, where high-consequence agricultural pathogens are used, to obtain network access. The PIV cards used at PIADC are 5.5×8.5 cm plastic polyvinyl chloride cards with an integrated gold microchip and magnetic strip covered by a laminated coating (Figure 1). The risk of PIV card contamination with high-consequence agricultural pathogens is considered to be extremely low because these pathogens are manipulated only within primary containment devices (eg, biological safety cabinets) in laboratory spaces where

PIV cards are permitted. In this study, we sought to validate the PIADC procedure for decontamination of PIV cards exiting biocontainment to meet PIADC operational requirements.

Virkon® S is a commercial disinfectant registered for use against both FMDV and SVDV by the US Environmental Protection Agency (EPA),⁹ and it is marketed as a broad-spectrum on-farm disinfectant for agricultural biosecurity (Lanxess). The active chemical ingredients of Virkon® S are potassium peroxydisulphate (21.41%) and sodium chloride (1.5%) with inorganic buffers. Virkon® S is the primary disinfectant employed at PIADC for decontamination in the high-containment laboratory. It is used to disinfect biological safety cabinets, work surfaces, and animal isolation rooms and as a boot dip when entering and exiting animal containment facilities. Seven commercial disinfectants are currently registered by the US EPA for use against FMDV.¹⁰ However, Virkon® S proved to be the least corrosive, most broad-spectrum, easy-to-use, and nonhazardous chemical option to meet PIADC operational requirements. Additionally, Virkon® S is the only commercial disinfectant that is registered by the US EPA for use against many other high-consequence agricultural pathogens used at PIADC, including African horse sickness virus,

Figure 1. Personal identity verification card, a smart card, showing viral inoculation sites as red spots.

African swine fever virus, classical swine fever virus, and vesicular stomatitis virus.^{9,10}

The current PIADC biosafety protocol for transfer of small, nonporous items (eyeglasses, CDs) out of BSL-3 biocontainment requires immersion in 1% Virkon® S for 30 seconds, followed by a water rinse, prior to personnel exiting via a 5-minute shower, and was the basis for this study. A 1% Virkon® S solution delivers 9.75% available chlorine, similar to household bleach,⁹ and requires a minimum 10-minute contact time, per label instructions. However, for routine disinfection of personal items such as PIV cards, we sought to evaluate efficacy at shorter contact times that are more practical for high-containment laboratory operations.

The goal of these studies was to evaluate the ability of 1% Virkon® S to disinfect PIV card surfaces intentionally spotted with high concentrations of both FMDV and SVDV, simulating accidental contamination of the PIV card. Studies were designed to evaluate viral inactivation under worst-case scenario parameters that included viral loads dried in the presence of an organic soil load and disinfectant solutions prepared in hard water and aged for 5 days. Relatively short disinfectant contact times were evaluated to support practical use in a laboratory environment.

METHODS

Virus Stocks and Cell Lines

FMDV strain A24/Cruzeiro/BRA/55 was obtained from the US Department of Homeland Security PIADC virus repository and amplified in LFBK α V β 6 cells (kindly supplied by M. LaRocco, US Department of Agriculture [USDA] Agricultural Research Service, PIADC).^{11,12} SVDV strain UKG 27/72 was amplified in MVPK cells, both of which were generously provided by the USDA Animal Plant Health Inspection Service, Foreign Animal Disease Laboratory, PIADC. Cells were propagated in complete Dulbecco's Modified Eagle's medium (cDMEM, Gibco, #12430054) supplemented with 10% fetal bovine serum, 1X antibiotic/antimycotic (Gibco, #15240062), and 1X sodium pyruvate (Gibco, #11360070) in T150 flasks and incubated at 37°C with 5% CO₂. At 72 hours postinfection, cell supernatants were clarified by centrifugation, aliquoted, and stored at -70°C. The initial viral stock concentrations were 8.29 and 8.23 log₁₀ TCID₅₀ (median tissue culture infective dose)/mL for FMDV and SVDV, respectively.

Soil Load Preparation

The soil load used in the quantitative carrier test is an American Society for Testing and Materials (ASTM) standard used to represent normalized protein levels found in bodily secretions.¹³ Briefly, solutions of 5.0% yeast extract (Cole Parmer, #BP142210), 5.0% bovine serum albumin (BSA) (Cole Parmer, #BP6711), and 0.4% bovine mucin

(Sigma, #M3895) were prepared using sterile phosphate buffered saline (PBS) and filtered through a 0.22- μ m filter under vacuum. Soil load component solutions were aliquoted as single-use portions and stored at -20°C until inoculum preparation.

Inoculum Preparation

The virus inoculum for each study was prepared immediately before spotting PIV cards on the morning of each test day from previously aliquoted and frozen single-use virus stocks. Briefly, virus stocks were supplemented to contain final concentrations of 0.35% yeast extract, 0.25% BSA, and 0.04% bovine mucin. To achieve this, 340 μ L of the virus suspension was combined with 25- μ L BSA stock, 35- μ L yeast extract stock, and 100- μ L bovine mucin stock, prepared as described above.

Disinfectant Preparation

Powdered Virkon® S (Pharmalac, #03010F) was dissolved in hard water to produce a 1% solution (pH ~2.5). Hard water was formulated according to the EPA guidelines (SOP No. MB-30-01),¹⁴ and final CaCO₃ concentrations were determined by titration with a Hach Total Hardness Test Kit (Hach #2063600). The target hardness was 375 mg CaCO₃/L, with an acceptable error margin of $\pm$ 5-10% (338-394 ppm). Disinfectant solutions were prepared 5 days in advance of the day of testing to simulate an aged formulation consistent with current PIADC biocontainment protocols that mandate weekly preparation of Virkon® S solutions.

PIV Card Inoculation

New, unused PIV cards were used as experimental coupon carriers. Briefly, 50 μ L of either the FMDV or SVDV inocula test suspension containing the organic soil load was pipetted onto the cards at 2 locations, the gold metallic and laminate plastic surfaces (Figure 1), and air-dried in a certified Class II biological safety cabinet at room temperature. Drying of the virus inoculum took approximately 1.5 hours. Virus-spotted PIV cards and controls were processed immediately after drying.

Quantitative Carrier Disinfection Assay

The effect of contact time on PIV card disinfection was determined by immersion of PIV cards in 1% Virkon® S for 1, 10, 30, or 60 seconds. Exposures consisted of dipping each card vertically using sterile plastic forceps into a 1% Virkon® S solution (500 mL), followed by a 1-second dip in a sterile deionized water bath (200 mL). Individual beakers of Virkon® S and water were used for each card to

ensure no cross-contamination occurred. For each study, 3 positive control groups were included, as follows: (1) a TCID₅₀ back-titration of the virus and soil load suspension to confirm the starting titer delivered to each card, (2) a drying control to assess the effect of drying on loss of virus infectivity, and (3) a dipping control to measure any loss of virus infectivity by physically immersing the PIV card for 60 seconds in a neutral liquid (water) without disinfectant (Table 1). Disinfection experiments were carried out in a certified Class II biological safety cabinet in BSL-3 containment. Two identical studies were conducted for each treatment to assess inter-assay variation. Each experimental contact time treatment group included 3 PIV card replicates per time point, and positive controls included 1 PIV card per treatment group.

Viral Recovery

Following immersion in Virkon® S and a water rinse, PIV cards were placed into individual sterile plastic boxes containing 10 mL of DMEM +2% FBS to neutralize any remaining Virkon® S and shaken for 10 minutes on an orbital shaker at room temperature to elute any remaining viral inoculum. FMDV eluates were serially diluted 10-fold from 10⁻¹ to 10⁻⁶ in cDMEM and plated in replicates of 8 wells per dilution onto 96-well microtiter plates seeded with adherent monolayers of LFBK_{αVβ6} cells. In order to increase the limit of detection (LOD), SVDV eluates were serially diluted from 10⁰ to 10⁻⁵ and plated onto adherent MVPK cell monolayers in either 96-well (Study 1) or 48-well (Study 2) plates. After 3 days of incubation, cell monolayers were scored for virus cytopathic effects (CPE), and the TCID₅₀ of each viral sample was calculated via the Reed-Muench method.¹⁵ The LOD was 2.0 log₁₀ for the FMDV studies, and 1.0 and 1.3 log₁₀ for the first and second SVDV studies, respectively. The assay LOD value was used to calculate the average recovery for samples with no CPE. In the repeat study for each virus, supernatants from all CPE-negative samples were incubated on the appropriate cell line for 2 additional blind passes (72 hours each) in 96-well plates.

During methods development, it was shown that DMEM +2% FBS effectively neutralized the chemical activity of Virkon® S at the assay use-dilution of 50 µL chemical: 10 mL neutralizer and did not induce cytotoxicity on the cell lines used (data not shown). Additionally, swabbing the surface of PIV cards resulted in recovery of <1% of the virus recovered from PIV cards after elution in DMEM, suggesting that the majority of infectious virus was eluted into the media during shaking and did not remain stuck to the card (data not shown).

Statistical Analyses

Mean, standard deviation, and 2-tailed *t*-tests were calculated using Excel 2013 (Microsoft).

RESULTS

Card Functionality

Prior to conducting the decontamination studies of FMDV and SVDV on PIV cards, the physical integrity and functionality of PIV cards was evaluated after exposure to Virkon® S. No adverse effects on PIV card structure or function were found after immersion into 2% Virkon® S for up to 10 hours (data not shown).

Recovery from Positive Control Groups

After combination with the soil load components, the average starting titers of undried FMDV and SVDV were 7.3 and 5.65 log₁₀ TCID₅₀/mL, respectively (Figure 2). Drying of the viral inocula onto card surfaces resulted in average losses in titer of 0.2 (from 7.3 to 7.1) and 0.4 (from 5.6 to 5.2) log₁₀ for FMDV and SVDV (Figure 2). Thus, recovery of dried FMDV and SVDV from PIV card surfaces was sufficient to determine virus inactivation values for FMDV (Table 2) and SVDV (Table 3) using CPE-based TCID₅₀

Table 1. Description and experimental significance of positive and negative control groups

<i>Control Group</i>	<i>Description</i>	<i>Significance</i>
Negative control	100 µL of DMEM + soil load dried on PIV card without virus; card inoculum was eluted in 10 mL media.	Demonstrates that soil load components and PIV card are not cytotoxic to cell lines
Back titration control	100 µL viral inoculum + soil load inoculated directly into 10 mL media, no drying.	Demonstrates starting titer of infectious virus inoculum prior to drying
Drying control	100 µL viral inoculum + soil load dried on PIV card and eluted in 10 mL media.	Demonstrates titer of recoverable infectious virus after drying of inoculum on card surfaces
Dipping control	100 µL viral inoculum + soil load dried on PIV card; card was immersed in water (60 seconds) and eluted in 10 mL media.	Demonstrates effect of mechanically dipping card in liquid on ability to recover virus without disinfectant exposure

Figure 2. Recovery of FMDV and SVDV from positive control and Virkon® S-exposed PIV cards after 1-, 10-, 30-, and 60-second contact times. Bars designate the geometric mean recovery ($\pm$ standard deviation, SD) for each virus across 2 studies. For Virkon® S-exposed cards, $n=6$ replicates (3 per study), and for each control group $n=2$ replicates (1 per study). Numbers above the bars indicate the number of PIV cards with detectable virus from 6 experimental replicates per group. Values for CPE-negative samples are reported as the assay limit of detection, which was $2.0 \log_{10} TCID_{50}/mL$ for FMDV and 1.0 and 1.3 for SVDV studies 1 and 2, respectively. ND=not done.

log-reduction calculations. The physical process of dipping cards contaminated with dried inoculum in water for 60 seconds resulted in average titer reductions of 1.6 and $1.3 \log_{10}$ for FMDV and SVDV, respectively (Figure 2). Starting titers of both FMDV and SVDV were sufficient

($>10^{-4} \log_{10} TCID_{50}/mL$) to meet the EPA virucidal efficacy requirements for chemical disinfectant registration testing in which a $4\text{-}\log_{10}$ reduction in infectious virus must be demonstrated.¹⁶ No CPE or cytotoxicity was observed in cells inoculated with eluates from negative control coupons.

Table 2. Recovery of foot-and-mouth disease virus from personal identity verification cards

Control	Study 1			Study 2		
Back titration control	7.3			7.3		
Drying control	6.7			7.5		
Dipping control	5.5			5.9		
Contact Time with Virkon® S	Study 1 Replicates			Study 2 Replicates		
	A	B	C	A	B	C
1 second	6.2	6.7	6.7	6.6	6.6	6.0
10 seconds	<2.0	<2.0	<2.0	3.5	<2.0	<2.0
30 seconds	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
60 seconds	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

Values are $\log_{10} TCID_{50}/mL$. Limit of detection (LOD) = $2.0 \log_{10} TCID_{50}/mL$.

Table 3. Recovery of swine vesicular disease virus from personal identity verification cards

Control	Study 1			Study 2		
Back titration control	5.3			5.8		
Drying control	4.3			6.1		
Dipping control	3.7			4.1		
Contact Time with Virkon® S	Study 1 Replicates			Study 2 Replicates		
	A	B	C	A	B	C
10 seconds	<1.0	2.6	1.1	1.7	<1.3	2.8
30 seconds	2.7	<1.0	<1.0	<1.3	<1.3	<1.3
60 seconds	<1.0	<1.0	<1.0	<1.3	<1.3	<1.3

Values are $\log_{10}$ TCID₅₀/mL. Limit of detection (LOD) = 1.0 $\log_{10}$ TCID₅₀/mL (Study 1), and 1.3 $\log_{10}$ TCID₅₀/mL (Study 2).

Virus Recovery After Exposure to 1% Virkon® S

For FMDV-spotted PIV cards, exposure to 1% Virkon® S for 1 second resulted in no decrease in FMDV titer. A 10-second contact time resulted in inactivation of FMDV on 5 of 6 PIV cards, with 1 PIV card having a residual FMDV titer of 3.5 $\log_{10}$ TCID₅₀/mL (Table 2). Contact times of 30 seconds and 60 seconds resulted in inactivation of FMDV below the assay LOD for all PIV cards (Table 2).

For SVDV-spotted PIV cards, an exposure time of 1 second was not tested due to the lack of inactivation in the FMDV study. A 10-second exposure resulted in SVDV inactivation on 1 of 6 PIV cards, with an average recovery of 1.74 $\log_{10}$ (Table 3, Figure 2). A contact time of 30 seconds inactivated SVDV on 5 of 6 PIV cards, and a 60-second contact time inactivated SVDV on 6 of 6 PIV cards. All inactivation studies included 3 replicates for each treatment at each contact time.

Because inter-assay variability was not statistically significant (2-tailed *t*-test, $P \geq 0.4$), data presented are the average of all 6 samples for a disinfectant time treatment group (Figure 2). In each repeat study, CPE-negative supernatants were blind-passaged on cells twice more (72 hours each), with no additional CPE-positive wells observed for either virus at contact times of 30 and 60 seconds.

DISCUSSION

Use of smart cards has increased in the past decade in response to increased security requirements for IT network and physical access controls. Current estimates from the Office of Management and Budget indicate that nearly 5 million PIV cards have been issued to federal employees and contractors.¹⁷ As the number of smart card users continues to increase to meet demands for improved security at federal, military, and hospital facilities, disinfection of card surfaces could be of interest in environments where contamination may occur.

Other options for 2-factor authentication in high-containment laboratories include use of centralized PIV

card readers and computer-derived credentials. However, PIADC determined that a simple step for decontaminating PIV cards during the biocontainment exit process was the most practical option for implementation, cost, and efficacy with minimal interruption to workflow. We believe this is the first reported study validating a decontamination process for the safe removal of smart cards from a high-containment laboratory.

The use of quantitative carrier assays is a widely accepted method to determine virucidal efficacy of chemical disinfectants for virus inactivation on the surface of nonporous materials.^{13,18} Our modified assay used the specific object of decontamination interest, the PIV card, as the coupon carrier and FMDV and SVDV to analyze a facility-specific biosafety process. Evaluating decontamination efficacy directly against the target pathogens used in the PIADC high-containment laboratory, compared to using viral surrogates or biological indicators that may differ in biological and physical characteristics, provided accurate and specific data to support institutional disinfection protocols that address the actual biosafety risk.

Virkon® S has been reported to inactivate FMDV and SVDV in quantitative carrier tests after a 10-minute contact time.^{19,20} However, little data exist regarding inactivation at shortened contact times. Our data indicate that exposure to a 5-day-old 1% Virkon® S solution for at least 30 seconds resulted in complete inactivation of FMDV, whereas 60 seconds of contact with Virkon® S was needed to inactivate SVDV. These contact times are significantly shorter than the 10-minute contact time recommended by EPA. Therefore, the results further validate current PIADC biosafety protocols as practical and effective.

The risk of PIV card contamination in biocontainment at PIADC is considered extremely low because of the use of engineering controls (biological safety cabinets) and other biosafety practices. This study was designed to model contamination levels well above those expected at PIADC to simulate a worst-case scenario of contamination. Although the scope of this study was limited to use of Virkon® S against 2 small, nonenveloped viruses, this method provides a framework for other facilities to evaluate other disinfectants

against pathogens specific to their needs. Studies designed to model worst-case contamination scenarios will ensure that institutional operational procedures effectively mitigate biorisks with confidence.

CONCLUSION

We developed a modified quantitative carrier test method using the specific object of interest as the coupon and target high-consequence agricultural pathogens as the test organisms, in order to validate disinfection procedures for PIADC-specific biosafety practices. We found that immersion of PIV cards in 1% Virkon® S for 60 seconds effectively inactivated dried FMDV and SVDV in the presence of a soil load. Because the disinfection process was effective against viral loads many times greater than anticipated for accidental unobserved laboratory contamination, this study validated the PIADC protocol for disinfection of PIV cards exiting BSL-3 biocontainment.

ACKNOWLEDGMENTS

We thank Drs. Barbara Kamicker, David Brake, and José Barrera for editing the manuscript and Colleen Kolb for testing PIV card functionality after chemical exposure. The US Department of Homeland Security Science and Technology Directorate (DHS S&T) funded an agreement with Leidos (HSHQDC-14-F-00035 [contract GS-23F-80006H]). This research was supported in part by an appointment of Justin Smith to the PIADC Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the US Department of Energy (DOE) and DHS S&T. ORISE is managed by ORAU under DOE contract number DE-AC05-06OR23100. DHS S&T, as the funding source, had no involvement in the study design; collection, analysis, and interpretation of data; writing of the report; or in the decision to submit the article for publication. All opinions expressed are the authors' and do not necessarily reflect the policies and views of DHS, DOE, or ORAU/ORISE.

REFERENCES

1. Agricultural Bioterrorism Protection Act of 2002; Biennial Review and Republication of the Select Agent and Toxin List; Amendments to the Select Agent and Toxin Regulations, Final Rule. *Fed Regist* 2017;82(12):6197-6210. <https://www.gpo.gov/fdsys/pkg/FR-2017-01-19/pdf/FR-2017-01-19.pdf>. Accessed July 19, 2018.
2. US Centers for Disease Control and Prevention. Federal Select Agent Program. Policy Statement: Application of the requirement for a "validated inactivation procedure" as used

- in the select agent regulations. April 6, 2017. https://www.selectagents.gov/policystatement_inactivation.html. Accessed July 19, 2018.
3. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*. 5th ed. <https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>. Accessed July 19, 2018.
4. Spaulding EH, Emmons EK. Chemical disinfection. *Am J Nurs* 1958;58(9):1238-1242.
5. Spickler AR. Technical Fact Sheet: Foot and mouth disease. Iowa State University, Center for Food Security and Public Health. Updated April 2014. http://www.cfsph.iastate.edu/Factsheets/pdfs/foot_and_mouth_disease.pdf. Accessed July 19, 2018.
6. Spickler AR. Technical Fact Sheet: Swine vesicular disease. Iowa State University, Center for Food Security and Public Health. Updated December 2017. http://www.cfsph.iastate.edu/Factsheets/pdfs/swine_vesicular_disease.pdf. Accessed July 19, 2018.
7. Bush GW. Homeland Security Presidential Directive 12: Policy for a common identification standard for federal employees and contractors. US Department of Homeland Security website. Updated August 19, 2015. <https://www.dhs.gov/homeland-security-presidential-directive-12>. Accessed July 19, 2018.
8. Lew JJ. Memorandum for the heads of executive departments and agencies. Executive Office of the President, Office of Management and Budget. February 3, 2011. <http://www.cac.mil/Portals/53/Documents/m-11-11.pdf>. Accessed July 19, 2018.
9. US Environmental Protection Agency. Master Label: Virkon® S EPA Reg. No. 71654-6. Accepted March 12, 2015. https://www3.epa.gov/pesticides/chem_search/ppls/071654-00006-20150312.pdf. Accessed July 19, 2018.
10. US Department of Agriculture, Animal and Plant Health Inspection Service. Potential pesticides to use against the causative agents of selected foreign animal diseases in farm settings. July 2017. https://www.aphis.usda.gov/animal_health/emergency_management/downloads/fad_epa_disinfectants.pdf. Accessed July 19, 2018.
11. LaRocco M, Krug PW, Kramer E, et al. A continuous bovine kidney cell line constitutively expressing bovine $\alpha\beta 6$ integrin has increased susceptibility to foot-and-mouth disease virus. *J Clin Microbiol* 2013;51(6):1714-1720.
12. LaRocco M, Krug PW, Kramer E, et al. Correction for LaRocco et al., A continuous bovine kidney cell line constitutively expressing bovine $\alpha\beta 6$ integrin has increased susceptibility to foot-and-mouth disease virus. *J Clin Microbiol* 2015;53(2):755.
13. ASTM E2197-17e1. Standard quantitative disk carrier test method for determining bactericidal, virucidal, fungicidal, mycobactericidal, and sporidial activities of chemicals. <https://doi.org/10.1520/E2197-17E01>. Accessed July 19, 2018.
14. US Environmental Protection Agency (EPA). Standard operating procedure for preparation of AOAC and OECD hard water and other diluents for preparation of antimicrobial products, SOP Number: MB-30-01. US EPA Office of Pesticide Programs Microbiology Laboratory, Fort Meade, MD. Revised February 3, 2017. <https://www.epa.gov/sites/production/files/2018-01/documents/mb-30-01.pdf>.

15. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. *Am J Hyg* 1938;27(3):493-497. <https://doi.org/10.1093/oxfordjournals.aje.a118408>. Accessed July 19, 2018.
16. US Environmental Protection Agency (EPA). *Product Performance Test Guidelines. OCSPP 810.2200: Disinfectants for Use on Hard Surfaces—Efficacy Data Recommendations*. EPA 712-C-07-074. US EPA Office of Chemical Safety and Pollution Prevention. 2012. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0150-0021>. Accessed July 19, 2018.
17. National Institute of Standards and Technology. Personal identity verification of federal employees and contractors. NIST Computer Security Resource Center. Updated July 17, 2018. <https://csrc.nist.gov/projects/piv>. Accessed July 19, 2018.
18. Organisation for Economic Co-operation and Development. *Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides Used on Hard Non-porous Surfaces*. Series on Testing and Assessment No. 187, Series on Biocides No. 6. June 21, 2013. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2013\)11&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)11&doclanguage=en). Accessed July 19, 2018.
19. Hole K, Ahmadpour F, Krishnan J, Stansfield C, Copps J, Nfon C. Efficacy of accelerated hydrogen peroxide® disinfectant on foot-and-mouth disease virus, swine vesicular disease virus and Senecavirus A. *J Appl Microbiol* 2017;122(3):634-639.
20. Bieker J. *Chemical Inactivation of Viruses* [doctoral dissertation]. Manhattan, KS: Kansas State University; 2006. <http://krex.k-state.edu/dspace/bitstream/handle/2097/226/JillBieker2006.pdf?sequence=1&isAllowed=y>.

Manuscript received March 13, 2018;

revision returned June 19, 2018;

accepted for publication July 3, 2018.

Address correspondence to:

Max V. Rasmussen, PhD

US Department of Homeland Security

Plum Island Animal Disease Center

PO Box 848

Greenport, NY 11944

Email: max.rasmussen@st.dhs.gov