Recovery and chemical disinfection of foot-and-mouth disease and African swine fever viruses from porous concrete surfaces

L.R. Gabbert¹, J.G. Neilan² and M. Rasmussen²

¹ Leidos, Plum Island Animal Disease Center, Greenport, NY, USA
² United States Department of Homeland Security, Science and Technology Directorate, Plum Island Animal Disease Center, Greenport, NY, USA

Abstract

Aims: Develop an effective laboratory method to consistently recover viral loads from porous concrete coupons sufficient for disinfectant efficacy testing. Investigate the role of concrete matrix pH on the recovery of foot-and-mouth disease virus (FMDV) and African Swine Fever virus (ASFV) from porous concrete. Compare parameters of FMDV and ASFV inactivation on porous and nonporous surfaces in quantitative carrier tests of a liquid chemical disinfectant.

Methods and results: Concrete test coupons were fabricated from commercial and industrial sources and carbonated by exposure to 5% CO₂ in a humidified incubator, lowering the matrix pH. Neither dried FMDV nor ASFV were recovered from high-pH concrete control coupons. Recovery of infectious virus from lower pH carbonated concrete was similar to stainless steel coupon controls. Exposure to the liquid disinfectant Virkon™ S inactivated FMDV and ASFV on porous concrete.

Conclusions: Concrete matrix pH had a greater impact than surface porosity on the ability to recover viable virus from unsealed concrete.

Significance and Impact of the Study: Concrete is commonly found in environments where virus decontamination is required. This study demonstrates a reproducible method to recover sufficient viral loads from porous concrete coupons to facilitate quantitative carrier testing. This method provides a basis for evidence-based validation testing of chemical disinfectants to inactivate pH-sensitive viruses on unsealed concrete.

Introduction

Effective disinfection of environmental surfaces contaminated with pathogenic microorganisms is crucial to prevent, respond and recover from infectious disease outbreaks. For high-consequence transboundary animal disease outbreaks due to an accidental or intentional release of agricultural pathogens, use of validated disinfection protocols ensures continuity of operations (USDA 2014, 2019). During such outbreaks, disease transmission and geographical spread are often attributed to contamination of food, fomites, facilities and transport vehicles with infectious agents. Effective cleaning and disinfection protocols result in destruction, inactivation or elimination of the infectious agent.

Demonstration of validated disinfection procedures on porous materials such as wood, cloth, paper and others have been historically difficult. Unsealed concrete is a porous material widely used in agricultural facilities, laboratories and food processing plants where environmental contamination with microorganisms may occur. Validated decontamination and disinfection methods and protocols for unsealed concrete surfaces are notably lacking (Krug et al. 2018). Quantitative efficacy testing of chemical disinfectants applied to porous, unsealed concrete is often hindered by insufficient recovery of viral...
loads from concrete controls, resulting in critical knowledge gaps. Potential reasons for poor recovery of viable virus from unsealed concrete include irreversible physical adhesion of virus to the porous concrete substrate and viral inactivation after contacting the substrate (Bieker 2006; Krug et al. 2018).

Quantitative carrier tests are commonly used to assess disinfection methods for inactivation of microorganisms dried on various materials representing environmental surfaces. These standardized tests are typically conducted using nonporous coupons such as stainless steel or glass (Sattar et al. 2003, Springthorpe and Sattar 2005, ASTM 2017), and success is dependent upon sufficient recovery of microorganisms from untreated, positive control coupons (Wyrzykowska-Ceradini et al. 2019). Insufficient recovery (<4-log10) of viable virus from control coupons precludes demonstrating the minimum 4-log10 reduction in infectious titre required for virucidal efficacy determination and subsequent product registration with the U.S. Environmental Protection Agency (US EPA 1981).

Reliable experimental data for disinfection of microorganisms on unsealed concrete are limited because low recovery from concrete controls often prevents quantitative comparisons of treatments. For example, Calfee and Wendling (2013) reported statistically significant differences in the recovery of vegetative bacteria from several porous coupon materials, with concrete and wood having the lowest recovery values. Additionally, Wood et al. (2010) were unsuccessful in recovering viable, highly pathogenic avian influenza virus from concrete coupons by multiple extraction methods.

Foot and mouth disease virus (FMDV) and African swine fever virus (ASFV) are high-consequence transboundary animal disease pathogens that cause severe economic consequences in endemic and epizootic countries (Thompson et al. 2002; Sánchez-Cordón et al. 2018). FMDV is a small, nonenveloped, positive-strand, RNA virus in the family Picornaviridae, and ASFV is a large, enveloped, DNA virus in the Asfarviridae family. Both pathogens are highly transmissible in domestic livestock populations, and may persist in the environment for months under favourable conditions (Botner and Belscham 2012; Bravo de Rueda et al. 2015).

Disinfection of ASFV and FMDV on nonporous carriers, including sealed concrete has been reported, but virus recovery from porous materials remains problematic (Krug et al. 2011, 2012, 2018; Gabbert et al. 2018). Bieker (2006) reported insufficient recovery of FMDV from untreated, porous concrete that resulted in inconclusive data for the chemical disinfectants tested. Krug et al. (2018) reported that concrete coupons having pH ≥ 10 were virucidal to high-titre FMDV and ASFV and suggested that efficacy tests could only be conducted after sealing the concrete to render it nonporous.

Potential mechanisms responsible for low viral recovery from unsealed concrete include high pH, which may inactivate viruses, and the possibility that viral particles may become irreversibly bound to the concrete matrix (Bieker 2006, USDA 2015, Wyrzykowska-Ceradini et al. 2019). The pH of freshly prepared concrete is highly alkaline, measuring approximately pH 13 (Grubb et al. 2007). Both FMDV and ASFV are inactivated at pH levels ≥10, presumably due to loss of the structural integrity of the FMDV capsid and ASFV envelope (Bachrach et al. 1957; Krug et al. 2011; Center for Food Security and Public Health (CFSPH) 2018; Kalmar et al. 2018).

Prolonged exposure to natural atmospheric and environmental conditions results in a gradual decrease in the pH of concrete over time. This process, termed carbonation, occurs due to the interaction of atmospheric carbon dioxide (CO2) with the hydration products of cement, resulting in the formation of calcium carbonate (CaCO3) and liberation of water: Ca(OH)2 + CO2 → CaCO3 + H2O (Hussain et al. 2016). However, the chemical reaction is largely dependent on relative temperature and humidity, and thus may take many years to complete under natural conditions (Chen et al. 2018).

In this study, we sought to determine the influence of concrete pH on the recovery of infectious FMDV and ASFV. We compared virus recovery from untreated, high-pH concrete, and from concrete coupons in which the pH had been lowered through accelerated gaseous carbonation in a laboratory environment. Following demonstration of sufficient virus recovery from carbonated, pH-adjusted concrete, we conducted quantitative virucidal efficacy tests with a U.S. EPA-registered disinfectant using unsealed concrete coupons and dried inocula of FMDV and ASFV.

Materials and methods

Virus stocks and cell lines

FMDV strains A24/Cruzeiro/BRA/55 and O1 Campos were amplified in a porcine kidney cell line expressing the FMDV integrin attachment receptors αV and β6 (LFBK-α5β6 cells), kindly supplied by M. LaRocco, U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS), Plum Island Animal Disease Center (LaRocco et al. 2013; LaRocco et al. 2015, Rodriguez, 2015). ASFV strain BA71V stocks were produced by amplification in Vero cells (ATCC® CCL-81™). Briefly, FMDV and ASFV were inoculated onto adherent monolayers of the appropriate cell line and cultured in Dulbecco’s Modified Eagle’s medium (DMEM; Gibco) supplemented with...
2% foetal bovine serum (FBS), 1X Antibiotic–Antimycotic (Gibco, 15240096), sodium pyruvate (Gibco™ 11360070), and incubated at 37°C with 5% CO₂ in T150 flasks. At days three (FMDV) and five (ASFV) postinfection, cells were subjected to three freeze/thaw cycles, and the cell supernatants clarified by centrifugation and stored as single-use aliquots at −70°C.

Stainless steel coupon preparation

AISI-brushed stainless steel disks type 430, with 1 cm diameter, 0.7 mm thickness (Pegen Industries Inc., Ontario, Canada) were used as nonporous, control-surface coupons. Stainless steel coupons were washed and degreased for 30 min in a 10% (w/v) solution of Alconox detergent, rinsed three times in deionized water and dried in a biological safety cabinet prior to sterilization by autoclaving for 45 min at 121°C.

Concrete coupon production

Three concrete formulations (Concretes A, B and C) were procured from separate sources and included two industrial mixes and one off-the-shelf ready-mix product. Concrete A coupons consisted of a silicate-based food-grade concrete (TurtleCrete, Reefball Inc., Nokomis, FL). Concrete B was an industrial-grade ready-mix concrete (4500 psi) with added plasticizer (Suffolk Cement Products, Inc., Calverton, NY). Concrete C was prepared in-house, based on mixing ratios indicated on the product label, after separating the coarse aggregate from an 80-pound bag of Sakrete High-Strength Concrete Mix (Lowes, 132022) and combining 438 g of the fine cementitious material with 100 ml of tap water. For all three concrete formulations, wet concrete slurry was poured into easy-release silicone ice cube trays to standardize size, shape and volume (one hundred and sixty 1-cm³ cubes/tray; Amazon.com) (Fig. 1). All cubes were air dried for a minimum of three days at room temperature before removal from the silicone trays. Individual concrete coupons were rinsed in deionized water and sterilized by autoclaving at 121°C for 15 min.

Concrete carbonation

Concrete coupons that received carbonation treatment were placed in a standard CO₂ incubator typically used for mammalian tissue culture (Sanyo model MCO-18AIC) for 7 days. The internal temperature was held constant at 37°C, and ambient air was continuously supplemented with CO₂ to obtain a final concentration of 5% CO₂ as measured by an infrared CO₂ sensor. Incubator humidity was regulated by natural vaporization of water in a humidifying pan. Relative humidity (rH) values, measured using a digital rH meter (ThermoPro TP-50), remained stable at 88% throughout the 7-day incubation. Carbonated coupons were sterilized by autoclaving, which did not change coupon surface pH (data not shown). As part of our methods development, we found that exposing concrete coupons to dry ice, sublimating CO₂, in a sealed container at room temperature for a period of three days, was ineffective at inducing carbonation, presumably due to the low temperature and lack of humidity.

pH Measurements

The pH of concrete matrices was measured using two methods. (i) Phenolphthalein (Sigma, 319236) is a pH indicator dye that remains colourless at pH values below 8.5, and becomes increasingly darker as pH increases.

Figure 1 Production of standardized concrete coupons by filling 1-cm³ silicone ice cube moulds with ready-mix concrete. Photo courtesy of Tod Barber, Reefball Inc., Nokomis, FL. [Colour figure can be viewed at wileyonlinelibrary.com]
above 8.5 (CID = 4764, https://pubchem.ncbi.nlm.nih.gov/compound/4764. Accessed 09DEC2019). To obtain an approximate qualitative pH value of the concrete surface before and after carbonation, 50 µl of a liquid phenolphthalein indicator solution depicting high initial pH of un-carbonated concrete. [Colour figure can be viewed at wileyonlinelibrary.com]

![Figure 2](image)

**Figure 2** Surface colour of dried concrete coupons before and after the addition of phenolphthalein indicator solution depicting high initial pH of un-carbonated concrete. [Colour figure can be viewed at wileyonlinelibrary.com]

Viral inoculum preparation

Briefly, 340 µl of a thawed, single-use frozen virus stock was mixed with 25 µl of BSA stock, 35 µl of yeast extract stock and 100 µl of bovine mucin stock, resulting in a final viral suspension containing 0.35% yeast extract, 0.25% BSA and 0.08% bovine mucin. These preparations were applied immediately to test coupons for quantitative carrier testing.

**Disinfectant preparation**

Virkon™ S (QC Supply, #340340) was prepared as a weight/volume solution by mixing powdered Virkon™ S in deionized water. Disinfectant solutions of 1, 2 and 5% were prepared immediately before use.

**Neutralization and cytotoxicity testing**

Disinfectant neutralization and cytotoxicity tests were performed in advance to rule out toxic effects on cell lines and viruses due to exposure to, or continued action of, the disinfectant after neutralization. Preparations of 2% Virkon™ S were tested. The EPA SOP MB-26-02 protocol (Environmental Protection Agency (EPA) 2017) was adapted for use with FMDV and ASFV in this study. Viral inocula were diluted to 10^2–10^3 infectious virus particles per 10 µl. At intervals of 30 ± 3 s, 10 µl of virus was added to flat-bottomed Nalgene vials containing either 10 ml of PBS (n = 3) or 10 ml of the neutralizer (DMEM + 2% FBS) (n = 3). Ten millilitres of the neutralizer was added to three additional vials containing 50 µl of the test chemical at 30-s intervals and allowed a 10-s contact time before 10 µl of the virus inoculum was added. Viral suspensions were serially diluted from 10^0 to 10^-3 and plated in replicates (6 wells/dilution) on 48-well plates. The cells were incubated for 3 days (FMDV) or 7 days (ASFV) and scored to detect cell cytopathic effects (CPE). CPE values were used to calculate the 50% Tissue Culture Infective Dose (TCID_{50}) endpoint titre (Reed & Muench 1938). Neutralizer effectiveness was determined by comparing the infectious virus titre of PBS controls and the vials containing the test chemical. A reduction of >0.5 log_{10} between the control group and the treatment containing the disinfectant was considered a failed neutralization. For cytotoxicity testing, we mixed 50 µl of disinfectant with 10 ml of neutralizer, added the suspension to both cell lines and observed cell monolayers for 3 days (LFBK-α,β/c) or 7 days (Vero) to determine if cell growth was impacted.

**Viral recovery and disinfection experiments**

All experiments were conducted based on the Organisation for Economic Cooperation and Development (OECD) method ‘Quantitative method for evaluating virucidal activity of microbicides used on hard
nonporous surfaces' (OECD 2013). To assess virus survival and recovery, 10 µl of the prepared virus inoculum was deposited on the stainless steel and concrete coupon surfaces and dried under desiccation in a class II biosafety cabinet for approximately 1 h. Individual coupons were transferred to Nalgene™ straight-side polypropylene jars with Screw Caps (Fisher Scientific, 11-815-10G), and 10 ml of neutralizer was added. Vials were vortexed for 30 s (±3 s) to dislodge virus from the coupon surfaces prior to quantitation of viable virus (as described above).

For the quantitative carrier disinfection tests, 50 (stainless steel) or 100 µl (concrete) of the test chemical was overlaid on the dried virus inoculum for either 5 or 10 min (±3 s) prior to elution in 10 ml of neutralizer. All eluates were serially diluted from 10⁻¹ to 10⁻⁶, and plated in replicates (n = 8) on adherent monolayers of LFBK-αβ₆ (FMDV) or Vero (ASFV) cells in 96-well plates. CPE was used to calculate log₁₀ TCID₅₀ ml⁻¹. Any eluates negative for infectious virus were blind-passaged three additional times to confirm virus inactivation. The assay lower limit of detection was 1.0 log₁₀ TCID₅₀ ml⁻¹, which was used to calculate a geometric mean for those samples. Samples were processed in replicates for untreated control coupons (n = 3) and for coupons receiving disinfectant (n = 4). The mean values include repeat tests from different test days.

**Results**

Concrete carbonation

The precarbonation pH values of crushed concrete coupons were Concrete A, pH 11.1; Concrete B, pH 12.4; and Concrete C, pH 12.6 (Table 1). Following CO₂ treatment, pH values for crushed coupons formulated with Portland cement-based concretes (Concretes B and C) were lower (pH 9.4 and 9.1, respectively) compared to their respective pretreatment values. The pH of the silica-based Concrete A remained unchanged at 11 post-CO₂ exposure (Table 1).

Qualitative analysis of coupon surface pH by application of the phenolphthalein pH-indicator dye demonstrated that pH values for carbonated Concretes B and C were below 8.5, whereas the surface pH of Concrete A remained unchanged (Fig. 3). Moreover, the surface pH of carbonated cubes of Concretes B and C remained stable for at least 12 months as shown by periodic re-application of phenolphthalein (data not shown).

Cross-section analyses of the pH of the concrete cube interior matrix showed that carbonation permeated throughout the entirety of the coupons Fig. 4). This is based on the absence of a colour change when phenolphthalein was applied to interior surfaces of Concretes B and C (Fig. 4), and by pH values obtained from crushed concrete coupons (Table 1). The pH of Concrete A remained stable at 11, and phenolphthalein treatment of interior surfaces produced a markedly dark colour change indicating pH values well above 8.5 (Table 1, Fig. 3).

Neutralization and cytotoxicity testing

Neutralization tests demonstrated that DMEM + 2% FBS neutralized 2% Virkon™ S within 10 s following addition to the disinfectant indicated by a <0.25 log₁₀ reduction in infectious viral titres between control reactions and those including the test chemical (Table 2). In previous studies, DMEM + 2% FBS neutralized 1% Virkon™ S (Gabbert et al. 2018).

No cytotoxicity was observed on LFBK-αβ₆ or Vero cell lines after neutralization of 1% and 2% Virkon™ S, as indicated by the absence of cell death and lack of changes in cell morphology after plating the neutralized test chemical. Use of 5% Virkon™ S elicited pH changes

![Table 1 pH values of crushed concrete coupons pre- and postcarbonation](https://example.com/table1.png)

| Concrete formulation | pH precarbonation | pH postcarbonation |
|----------------------|-------------------|--------------------|
| A                    | 11.1              | 11.0               |
| B                    | 12.4              | 9.4                |
| C                    | 12.6              | 9.1                |

**Figure 3** Colour of concrete coupons after application of pH-indicator dye phenolphthalein showing effect of carbonation on surface pH. Column 1: initial appearance of un-carbonated concrete coupons with no phenolphthalein dye. Column 2: un-carbonated concrete coupons after phenolphthalein application all showing surface pH > 8.6. Column 3: Carbonated concrete coupons after phenolphthalein application with Concrete A showing no noticeable change in pH, and Concretes B and C showing lower pH (<8.5) after incubation with gaseous CO₂ (5%) with humidity at 37°C for 1 week. [Colour figure can be viewed at wileyonlinelibrary.com]
in the culture media and cell death in the 10⁰ wells of serial  
dilution. However, no cytotoxicity was observed  
after dilution to 10⁻¹, allowing for inclusion in the disinfectant  
testing assays.

Phase I: virus recovery experiments

Comparison of virus recovery from dried and undried (wet) inocula on nonporous stainless steel coupons indicated virus losses ranging from 0.78 to 1.3 log₁₀ due to drying (Table 3). No viable FMDV or ASFV were recovered from uncarbonated, non-pH-adjusted concrete coupons, as evidenced by the lack of CPE in all samples tested. For pH-adjusted carbonated concrete coupons (Concrete types B and C), viable virus was recovered at levels comparable to, and sometimes better than, recovery from stainless steel controls (Table 3).

Concrete A, the silica-based product, was resistant to both surface and internal pH changes following CO₂ exposure, and functioned as a high-pH carbonation control. We hypothesize this is due to the increased silica content and lack of availability of calcium hydroxide to participate in the carbonation reaction, as explained by Wang and Lee, 2008. FMDV A24 virus was recovered at low levels from Concrete A coupons (n = 1/6), whereas infectious FMDV O1 Campos and ASFV were not detected.

All three viruses were recovered from carbonated Concrete C (Sakrete brand concrete mix) with FMDV O1 Campos titres exceeding recovery from stainless steel controls (Table 3). This result prompted the use of Concrete C coupons for all subsequent virus disinfection experiments.

Phase II: Virus disinfection experiments

Initial quantitative carrier disinfection experiments were conducted with FMDV A24 and ASFV BA71V on stainless steel and pH-adjusted Concrete C with Virkon™ S using a 5-minute contact time. Subsequent experiments included a 10-minute contact time for ASFV because the 5-min contact time was insufficient to completely inactivate ASFV. A FMDV A24 titre of >5 log₁₀ TCID₅₀ ml⁻¹ was recovered from control stainless steel and pH-adjusted concrete coupons (Table 4). Disinfection with 1% Virkon™ S on stainless steel resulted in a complete kill on all eight coupons (two test days). Disinfection on pH-adjusted Concrete C with 1, 2 and 5% Virkon™ S also resulted in complete virus inactivation based on the absence of detectable CPE in all replicates. Two additional blind passages of FMDV-negative eluates resulted in no additional positive samples.

An average of 3.2 (±0.2 SD) log₁₀ TCID₅₀ ml⁻¹ infectious ASFV was recovered from untreated stainless steel and pH-adjusted Concrete C controls (Table 5). Despite this lower initial starting titre compared to FMDV, infectious ASFV was detectable on 25% (1/4) of the stainless steel coupon control replicates and on all four concrete coupons after a 5-min contact time with 1% Virkon™ S. Following extension of the contact time to 10 min (per product label recommendation), ASFV was completely killed on stainless steel using 1% or 2% Virkon™ S. However, 75% (3/4) of the replicates were CPE positive when 1% Virkon™ S was applied to concrete coupons. Exposure to 2% Virkon™ S resulted in no initial detection of live virus. However, 25% (1/4) of the negative eluates were CPE positive following three blind passages in the innocuity test, suggesting a very low level of viable ASFV remained on one of the test coupons. Five per cent Virkon™ S eliminated ASFV on all replicates on carbonated Concrete C.

Table 2 Neutralization testing results for FMDV and LFBK-αVβ6 cells and ASFV and Vero cell with 2% Virkon™ S

| Test chemical | Neutralizer | Virus       | Cell line | Cytotoxicity | log₁₀ reduction |
|---------------|------------|-------------|-----------|--------------|-----------------|
| 2% Virkon™ S  | DMEM + 2% FBS | FMDV A24   | LFBK-αVβ6 | No           | −0.10           |
| 2% Virkon™ S  | DMEM + 2% FBS | ASFV BA71V | Vero      | No           | 0.22            |

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Transboundary animal disease viruses pose significant risks to food security and national economies. Determining effective chemical disinfection methods on surfaces commonly found in livestock and food-processing facilities is critically important to prevent and recover from outbreaks.

Most efficacy tests for liquid chemical disinfectants use nonporous surfaces or liquid suspensions to enable recovery of sufficient numbers of target microorganisms to calculate the extent of inactivation (OECD 2013; Environmental Protection Agency (EPA) 2016; ASTM International 2017). The use of standardized quantitative carrier assays to validate disinfection methods is challenging for porous materials that may inactivate or otherwise prevent recovery of viable target microorganisms from the test surface. Disinfectant efficacy data for porous materials such as concrete have been limited due to poor recovery of test microbes from the concrete matrix (Bieker 2006; Krug et al. 2018). The current study demonstrates a simple, cost-effective and reproducible method to recover sufficient viral loads from porous concrete coupons to enable quantitative evaluation of disinfectant efficacy. From a functional standpoint, the performance of the porous, carbonated concrete testing coupons was equivalent to that of nonporous stainless steel. The carbonated concrete coupons withstand repeated rounds of

### Table 3 FMDV and ASFV recovery from test coupons

| Treatment Group          | FMDV A24 | FMDV O1 Campos | ASFV BA71V |
|--------------------------|----------|----------------|------------|
| Undried virus*           | 6.09 ± 0.50 (15/15) | 5.07 ± 0.43 (4/4) | 3.95 ± 0.70 (11/11) |
| Stainless steel          | 5.31 ± 0.31 (15/15) | 4.24 ± 0.52 (7/7) | 2.65 ± 0.71 (13/13) |
| Uncarbonated concrete    | A <1.0 ± 0.0 (0/6)  | <1.0 ± 0.0 (0/3)  | <1.0 ± 0.0 (0/3)  |
|                          | B <1.0 ± 0.0 (0/6)  | <1.0 ± 0.0 (0/3)  | <1.0 ± 0.0 (0/3)  |
| Carbonated concrete      | A 0.23 ± 0.57 (1/6) | <1.0 ± 0.0 (0/3)  | <1.0 ± 0.0 (0/3)  |
|                          | B 4.45 ± 0.94 (6/6) | 4.54 ± 0.38 (3/3) | 2.19 ± 0.37 (3/3) |
|                          | C 4.93 ± 0.70 (9/9) | 5.57 ± 0.37 (3/3) | 2.66 ± 0.79 (9/9) |

Values reported as log10 TCID50 ml⁻¹ ± standard deviation (number of positive coupons/total).

*Undried virus control group was included to show the impact of drying on starting titres. Starting volumes were 10 µl of inoculum for both dried and undried virus.

### Table 4 FMDV A24 inactivation on stainless steel and concrete following a 5-min exposure to Virkon™ S

| Treatment group                   | Infectious virus recovered (log10 TCID50 ml⁻¹) ± SD | Number of positive coupons/total |
|-----------------------------------|-----------------------------------------------|---------------------------------|
| Untreated controls                |                                               |                                 |
| Undried virus                     | 5.81 ± 0.13 (6/6)                            |                                 |
| Dried Virus on Stainless Steel    | 5.27 ± 0.36 (6/6)                            |                                 |
| Dried Virus on Concrete           | 5.09 ± 0.29 (6/6)                            |                                 |
| Stainless steel                   |                                               |                                 |
| 1% Virkon                         | <1.0 ± 0.00 (0/8)                            |                                 |
| Carbonated concrete               |                                               |                                 |
| 1% Virkon                         | <1.0 ± 0.00 (0/8)                            |                                 |
| 2% Virkon                         | <1.0 ± 0.00 (0/4)                            |                                 |
| 5% Virkon                         | <1.0 ± 0.00 (0/8)                            |                                 |

### Table 5 ASFV BA71V inactivation on stainless steel and concrete following 5- and 10-min exposure to Virkon™ S

| Treatment Group | 5-minute contact | 10-minute contact |
|-----------------|------------------|-------------------|
|                 | Infectious virus recovered (log10 TCID50 ml⁻¹) ± SD | Number of positive coupons/total |
| Controls        | 4.56 ± 0.59 (3/3) | 3.86 ± 0.13 (3/3) |
| Dried virus     | 3.34 ± 0.3 (3/3)  | 3.2 ± 0.18 (3/3)  |
| Dried virus on concrete | 3.2 ± 0.2 (3/3)  | 2.93 ± 0.35 (3/3) |
| Stainless steel |                                               |                                 |
| 1% Virkon       | <1.12 ± 0.34 (2/3) | <1.0 ± 0.00 (0/4) |
| 2% Virkon       | n.d.              | <1.0 ± 0.00 (0/4) |
| Carbonated concrete |                                               |                                 |
| 1% Virkon       | 1.84 ± 0.71 (4/4)  | 1.25 ± 0.5 (3/4)  |
| 2% Virkon       | <1.0 ± 0.00 (0/4)  | <1.0 ± 0.00 (0/4) |
| 5% Virkon       | <1.0 ± 0.00 (0/4)  | <1.0 ± 0.00 (0/4) |

1098
CO2 concentration (Saetta et al. 2003; Chen et al. 2016). Thus, it should not be assumed that survival of pH-sensitive microorganisms is less probable on relatively new concrete construction, or that older buildings are more susceptible to contamination. For example, as part of the methods development for the current study, we measured pH values >12 from bores of 60-year-old concrete laboratory flooring (data not shown).

Accelerated carbonation of the concrete coupons enabled quantitative carrier efficacy tests with Virkon™ S, as demonstrated by high-level virus recovery and inactivation on the pH-adjusted, porous concrete test coupons. Virkon™ S was chosen as the test disinfectant in this study as it is currently registered by the U.S. EPA as a broad-spectrum disinfectant and virucide effective against both FMDV and ASFV at a concentration of 1% and a contact time of 10 min (EPA Reg. No. 39967-137; EPA 2019). Our results demonstrated that Virkon™ S was equally effective against FMDV A24 on pH-adjusted concrete as on stainless steel after 5 min. Previous studies with Virkon™ S reported rapid FMDV inactivation (Gabbert et al. 2018), and the current study is the first to report ASFV inactivation at shortened contact times with Virkon™ S.

Our results demonstrated that ASFV was slightly more resistant than FMDV to inactivation with Virkon™ S because a longer contact time was required on stainless steel, and a higher concentration of Virkon™ S (2–5%) was necessary on concrete, despite a lower starting titre of ASFV. These results also suggest that there may be slight differences in disinfectant activity when applied to porous concrete vs stainless steel.

In conclusion, we demonstrated recovery and disinfection of both a large enveloped (ASFV), and a small encapsidated (FMDV), virus from pH-adjusted porous concrete. Further studies are needed to determine if this method is suitable for other pathogenic microorganisms or disinfectants. The method reported here may enable future validations of virucidal efficacy for liquid disinfectants on unsealed concrete through quantitative carrier testing.

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Conflict of interest

The authors report that no conflicts of interest exist.

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