Persistence of Bacteriophage Phi 6 on Porous and Non-Porous Surfaces; Potential for use as Ebola or Coronavirus Surrogate

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Running Head: Ebola Surrogate Phi 6 Persistence on Hospital Surfaces

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ABSTRACT (250 words) The infection of healthcare workers during the 2013-2016 Ebola outbreak raised concerns about fomite transmission. In the wake of the Coronavirus Disease 2019 (COVID-19) pandemic, investigations are ongoing to determine the role of fomites in coronavirus transmission as well. The bacteriophage Phi 6 has a phospholipid envelope and is commonly used in environmental studies as a surrogate for human enveloped viruses. The persistence of Phi 6 was evaluated as a surrogate for EBOV and coronaviruses on porous and nonporous hospital surfaces. Phi 6 was suspended in a body fluid simulant and inoculated onto 1 cm² coupons of steel, plastic, and two fabric curtain types. The coupons were placed at two controlled absolute humidity (AH) levels; a low AH of 3.0 g/m³ and a high AH of 14.4 g/m³. Phi 6 declined at a slower rate on all materials under low AH conditions with a decay rate of 0.06 log₁₀PFU/d to 0.11 log₁₀PFU/d, as compared to the higher AH conditions with a decay rate of 0.65 log₁₀PFU/h to 1.42 log₁₀PFU/d. There was a significant difference in decay rates between porous and non-porous surfaces at both low AH (P < 0.0001) and high AH (P < 0.0001). Under these laboratory-simulated conditions, Phi 6 was found to be a conservative surrogate for EBOV under low AH conditions, in that it persisted longer than Ebola virus in similar AH conditions. Additionally, some coronaviruses persist longer than phi6 under similar conditions, therefore Phi6 may not be a suitable surrogate for coronaviruses.

IMPORTANCE (150 words) Understanding the persistence of enveloped viruses helps inform infection control practices and procedures in healthcare facilities and community settings. These data convey to public health investigators that enveloped viruses can persist and remain infective on surfaces, thus demonstrating a potential risk for transmission. Under these laboratory-simulated western indoor hospital conditions, Phi 6 was used to assess suitability as
a surrogate for environmental persistence research related to enveloped viruses, including EBOV and coronaviruses.

**KEYWORDS** Ebola, Phi 6, surface persistence, enveloped viruses, surrogate

**INTRODUCTION** Ebola virus (EBOV) is an enveloped RNA virus in the family *Filoviridae*, which also includes Marburg virus and Cueva virus. Ebola Virus Disease (EVD) is a rare but deadly disease with a case fatality rate around 50% (ranging from 25% to 90% based on outbreaks from 1976 to 2015) according to the World Health Organization (WHO) (1). The on-going outbreak that started in 2018, mainly occurring in Democratic Republic of Congo (DRC) and spilling over into Uganda, is the second largest with 2,997 cases and 1,998 deaths as of August 27, 2019 (2). The largest and deadliest EBOV outbreak on record (2013-2016) had more than 28,000 cases and over 11,000 deaths globally (3). The outbreak directly impacted the United States when, in 2014, a healthcare worker in Texas tested positive for EBOV after caring for an infected patient (4-6). Recorded cases from past and present outbreaks show that EBOV hospital transmission is a global concern as highlighted in a review of HCW infections by Selvaraj *et al.* (6), which details occupational exposure increasing transmission risk to healthcare workers.

Coronaviruses are also enveloped RNA viruses, belonging to the family *Coronaviridae*. Human coronaviruses are responsible for some common colds, but in 2002 SARS-CoV-1 emerged from Guangdong province, China as the first known deadly coronavirus (7) resulting in 8,422 illnesses and 916 deaths. In 2012 the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Outbreak, believed to have originated from Saudi Arabia, caused 858 deaths across
27 countries (8). Coronavirus disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) emerged from Hubei province, China (late 2019) and spread rapidly around the world being declared a pandemic by the WHO in March 2020 (9). Fomites were thought to have played a role in transmission of SARS-CoV-1 (10, 11), and though SARS-CoV-2 is thought to be primarily spread by aerosols, investigations are underway to determine if fomites also contribute to transmission. Emerging data suggests SARS-CoV-2 can persist for days on surfaces (12), though the influence of environmental factors still need to be explored.

The Centers for Disease Control and Prevention (CDC) recommends a combination of measures to prevent transmission of EVD in hospitals and these recommendations have been adapted for SARS-CoV-2 as well. The recommendations include patient isolation and record keeping, proper personal protection equipment (PPE) and correct use of PPE, dedicated equipment, limited use of sharps, avoiding aerosol generating procedures, hand hygiene, and monitoring potentially exposed personnel and visitors for signs and symptoms (13, 14). Further guidance from the CDC covers environmental infection control beyond PPE to include disinfectant use, routine cleaning, how to handle soiled surfaces and textiles, and how to transport or dispose of contaminated items and waste (15, 16).

The WHO offers similar guidance on PPE (including proper donning and doffing), infection prevention and control, hand hygiene, and management of wastes (1, 17, 18). Despite implementation of these best practices, transmission of Ebola from patient to healthcare worker continued, as documented by at least four reported cases in the fall of 2018 (19) in the United States of America. There have also been documented healthcare worker transmission of
SARS-CoV-1 and MERS (20) These transmission events highlight the need to understand the persistence of EBOV and other pathogenic enveloped viruses on fomites, and the role of fomites in transmission especially in the presence of body fluids (21, 22).

EBOV is a select agent and requires a Biosafety Level (BSL)-4 laboratory and specialized PPE to prevent potential life-threatening exposure. SARS-CoV-1 and SARS-CoV-2 are also labeled as select agents and require a BSL-3 laboratory in order to culture and conduct environmental persistence, sampling or disinfection studies in which working with live virus is required. For safety concerns, researchers have incorporated other methods to avoid handling and propagating this virus. Some have opted for the molecular detection of viral RNA to demonstrate potential transmission in the hospital environment (22, 23). Surrogate viruses have historically been used for transmission studies related to healthcare practices and can be employed as surrogates for viral persistence. Bacteriophage Phi 6, a member of the family Cystoviridae, was previously used as a surrogate for EBOV, influenza, coronavirus (SARS-1), Venezuelan equine encephalitis virus, and other pathogenic enveloped viruses (24-29).

Casanova et al. (30, 31) used Phi 6 as an Ebola surrogate to demonstrate transference to healthcare worker hands and scrubs during the PPE doffing procedures. However, data regarding the persistence of EBOV or its surrogates in the healthcare environment is limited. This study evaluated the persistence of Phi 6 in the presence of artificial test soil (ATS) as a potential surrogate for EBOV or Coronaviruses, at two absolute humidity (AH) conditions, on four potential fomites: non-porous steel (SS) and plastic (PL), and two types of porous hospital curtain fabrics.
RESULTS At the lower AH (3.0 g/m³) on SS, Phi 6 persisted >76 days (d) with 4.08 log₁₀ reduction (inoculum of 2.5 X 10⁴ plaque forming units [PFUs]) and there was no detectable infective phage by 102 d (Table 1). Phi 6 persisted on PL at the same AH up to 77 d with 4.20 log₁₀ reduction (inoculum of 2.4 X 10⁴ PFUs) and there was no detectable phage by 78 d. The statistical model, using a least square method in SAS v9.4 (Cary, NC), projected Phi 6 persistence until 72 days on SS and 70 days on PL (Figure 1). The model predicted a decay rate of 0.06 log₁₀/d for both SS and PL, with r² = 0.88 and 0.96, respectively at AH 3.0 g/m³ (Table 1). The projected data has a uniform standard error of +/- 0.04 log₁₀ PFUs/ml. There was no difference, by analysis of variance and the F statistic, in decay rates between SS and PL at the low AH (3.0 g/m³) conditions.

At the higher AH (14.4 g/m³) on SS, Phi 6 persisted >54 hours (h) with 3.33 log₁₀ reduction (inoculum of 5.2 X 10³ PFUs) and there was no infective phage by 77 h (Table 1). Phi 6 persisted on PL at the same AH up to 72 h with 3.68 log₁₀ reduction (inoculum of 1.9 X 10⁴ PFUs) and there was no detectable infective phage by 73 h. The model projected Phi 6 persisting until 56 h on SS and 71 h on PL (Figure 2). The model predicted a decay rate of 1.42 log₁₀/d (r² = 0.90) for SS and 1.09 log₁₀/d (r² = 0.91) for PL (Table 1). The projected data has a uniform standard error of +/- 0.14 log₁₀ PFUs/ml. There was significant difference in decay rates between SS and PL (P = 0.001) at the higher AH. Overall, Phi 6 persisted longer on SS and PL surfaces at the lower AH of 3.0 g/m³ (no detectable Phi 6 at 78 d to 102 d), as compared to the higher AH of 14.4 g/m³ (no detectable Phi 6 at 73 h to 77 h).
Phi 6 persistence on porous treated and untreated curtains (TC and UC) at the low AH (3.0 g/m³) conditions was >35 d with 5.40 log₁₀ reduction (inoculum of 2.3 X 10⁵ PFUs) and 5.27 log₁₀ reduction (inoculum of 2.2 X 10⁵ PFUs), respectively, and no Phi 6 was detectable at 42 d for either curtain types (Table 2). The model projected Phi 6 persisting until 35 d in the low AH conditions (Figure 1). The model of persistence on TC and UC predicted decay rates of 0.11 log₁₀/day for TC (r² = 0.72) and 0.10 log₁₀/day (r² = 0.71) for UC (Table 2). The projected data has a uniform standard error of +/- 0.04 log₁₀ PFUs/ml. There was no significant difference in the decay rates of the curtain types in low AH conditions (P = 0.200).

Phi 6 persistence on porous curtains (TC and UC) at high AH (14.4 g/m³) conditions decreased drastically compared to the low AH results for TC and UC. Time to no detection decreased from days to hours, where persistence on TC was 6 h with a 4.91 log₁₀ reduction (inoculum of 3.3 X 10⁶ PFUs) and on UC was 5 h with a 5.19 log₁₀ reduction (inoculum of 1.7 X 10⁶ PFUs) (Table 2). The model projected Phi 6 persisting until 9 h on TC and until 7 h on UC in the high AH conditions (Figure 2). The model predicted decay rates of 0.65 log₁₀/h for TC (r² = 0.71) and 0.71 log₁₀/h (r² = 0.49) for UC (Table 2). The projected data has a uniform standard error of +/- 0.15 for TC and +/- 0.14 log₁₀ PFUs/ml for UC. The difference between curtain types was not significant (P = 0.500) under high AH conditions.

Overall, bacteriophage Phi 6 in body fluid simulant persisted longer when held at the low AH of 3.0 g/m³ (35 d to 102 d) as compared to the higher AH of 14.4 g/m³ (5 h to 77 h) regardless of surface and material type (Table 1, 2). With respect to decay rates, Phi 6 declined slower on all materials under low AH (3.0 g/m³) conditions (0.06 log₁₀/d to 0.11 log₁₀/d).
compared to the higher AH (14.4 g/m³; 0.65 log₁₀/h to 1.42 log₁₀/d; Table 1,2). There were significant differences in decay rates between porous and non-porous surfaces at both low AH (P < 0.0001) and high AH conditions (P < 0.0001).

DISCUSSION

Phi 6 is an enveloped bacteriophage and was chosen for this study because it has been used as a surrogate for persistence of other enveloped viruses such as influenza, coronavirus and Venezuelan equine encephalitis virus (24, 26, 28). Using a non-pathogenic surrogate removes the need for resources associated with a BSL-3 or BSL-4 agent and makes the research procedures accessible to more laboratories. This current work demonstrated that the persistence of Phi 6 was similar to the published reports of EBOV (32-36) human respiratory viruses (25, 37), and coronavirus (38, 39) in that the phage persisted longer in colder temperatures and at lower relative and absolute humidity.

To date, two studies have evaluated EBOV persistence and found variability based on temperature, humidity, and substrate (32, 40). Persistence was also shown to vary between species of EBOV, Sudan EBOV and Zaire EBOV, and between variants Makona-C05 and Yambuku-Mayinga (21, 41, 42). Bausch et al. (21) found the risk of infection from fomites to be low when working with the Sudan EBOV, where only 2 of 33 surface swab samples taken daily in a Ebola isolation ward in Uganda were positive by PCR detection only, no samples were culture positive. In contrast, Bibby et al. found that the Zaire EBOV survived for over 14 days on glass and plastic in guinea pig sera held at 4°C (41), demonstrating the role of both temperature and strain in survival. Schuit et al. found differences between Makona-C05 and Yambuku-
Mayinga variants with the Mak-C05 variant remaining viable for longer in hospital room conditions (42).

Phi 6 was shown here in the current study to be a conservative surrogate for EBOV in a laboratory-simulated western hospital room condition of 3.0 g/m³ AH, persisting longer than the Makona-C05 variant (AH=3.3 g/m³) with decay rates of 0.06/d and 0.79/d respectively (Table 1). Due to different conditions, persistence comparisons between the current Phi 6 study and the Schuit et al. 2016 Ebola work at the higher AH was not possible; Phi 6 was evaluated at an AH of 14.4 g/m³ and EBOV was evaluated at an AH of 8 g/m³ or 25.8 g/m³ (42).

Interestingly, contrary to the trend we report with Phi 6 and studies seen with MERS-CoV (39), Schuit et al. demonstrated increased survival of Ebola at the higher AH of 25.8g/m³ (28°C, 90%RH) when deposited in dried blood as compared to his lower AH conditions tested.

A controlled laboratory study of SARS-CoV-1 and SARS-CoV-2 investigated persistence on steel and plastic, and found that both viruses behaved similarly (12). When conditions were held at AH 7.4 to 8.4 (21-23°C and 40% RH), no infective virus was detected by 72 and 96 h respectively (Table I). Chin et al. (43) demonstrated that SARS-CoV-2 persisted for 7 days at AH 12.7 (22°C, 65% RH). These conditions are close to our AH 14.4, in which persistence of Phi6 was observed for only 3 days, suggesting Phi6 may not be a suitable surrogate for SARS-CoV-2.

Additionally, a model based on testing at several humidity and temperature conditions predicted that SARS CoV-2 would persist for 4.4 d on steel and plastic, as suspended in an artificial saliva, and held at 14.4 AH (26°C, 57%RH), one of the same conditions we tested phi 6, though the model would not extend to the lower AH condition (44). Our work reported Phi 6...
persistence for 3 d under these conditions, a significantly shorter period, though the matrices were different. This adds evidence to the Chin et al. (45) results that Phi6 may not be a suitable surrogate for SARS-CoV-2 when persistence is being studied.

MERS-CoV was less persistent than Phi6 under close but not exactly the same conditions, surviving only to days 3 and 1 at low (6.9) and high (26.1) AH, respectively (39). Our study showed significantly greater persistence of Phi6 (78 to 102 days) at lower AH (3.0) than was tested for MERS-CoV. At the highest AH conditions tested, 14.4 for Phi6 and 26.1 for MERS-CoV, persistence for Phi6 was 6 h, while MERS-CoV was 24 h. Whether these differences can be explained by environmental test conditions, surface characteristics, or organism structure differences remains to be explored, but little parallel is seen between the studies.

Other Coronaviruses tested at AH 10.1-13.8 on aluminum persisted for even less time; HCV 229 persisted 12 h and HCV OC43 3 h (46) (Table 1). Persistence declines with increasing AH for Phi 6, but the literature does not reveal this trend for coronaviruses.

An integral data gap for enveloped viruses is the risk of transmission under various humidity and temperature conditions. The two temperature and humidity combinations applied in this study were upper and lower healthcare facility extremes. As related to EBOV and Coronaviruses, the upper extreme might be found in a tropical setting without adequate air conditioning, as seen in Liberia and Sierra Leone during the 2014-2016 epidemic, was 27.4°C (47). The lower extreme was chosen as a setting common in western healthcare facilities. Environmental temperature along with relative humidity (RH) were used to calculate the AH frequently referenced in the literature. AH is the measure of the water vapor in the air regardless of temperature, while RH is the ratio of the concentration of water vapor to the...
maximum possible concentration at a given temperature. Research on both Phi 6 and influenza have shown that AH may be more important than RH in virus infectivity (36, 48, 49) and the role of AH may be linked to changes in the viral envelope (50-53). Shaman and Kohn reported that influenza survival is more dependent on the water vapor in the air (AH) than how close the air is to saturation (RH) (49). Prussin et al. came to the same conclusion for Phi 6 when using a multiple regression analysis, showing that AH is a better predictor of virus infectivity (36). The role of AH in survival and infectivity may be linked to changes in the viral envelope during desiccation (50-53), and the protective effects of proteins upon concentration (54). One study noted a drop in infectivity between 60 and 80% RH at 25° and 37°C (AH 14.4 – 40 g/m³) (36), and similar findings were seen in an influenza persistence study (54), though these RH and AH conditions were higher than those tested here. Healthcare environments vary around the world and more information is needed to understand what influences persistence of enveloped viruses.

Damage from environmental conditions (e.g. AH, surface type, and matrices) to the viral envelope impact infectivity and persistence. Mateu et al. (55) suggests that repeated disruption of the capsid envelope can cause irreparable damage. Casanova et al. (50) suggest that viral inactivation is due to structural damage during desiccation, occurring at the air-water interface of the viral envelope. Persistence and infectivity of EBOVs have been shown to be influenced by the suspension liquid (blood, serum, cell culture media) and surface type, such as plastic and metal (non-porous), as well as curtain material (porous) (33, 35). Blood, mucus, stool, and other body fluids can provide protection from envelope structural damage due to desiccation (24, 41). In the hospital environment, Bausch et al. (21) detected EBOV by reverse
transcription polymerase chain reaction (RT-PCR) only upon visibly contaminated surfaces that were bloodstained. The transmission pathway of infected blood, and the protective nature of the blood matrix as shown by Fischer et al. (32) led to the use, in the current study, of ATS as a blood simulant, as it contains proteins, hemoglobin, and carbohydrates. Though testing only continued for 72 h, Wood et al. demonstrated better persistence of Phi 6 when suspended in blood diluent than in PBS (Table 1), and that the influence of the matrix overshadowed the influence of the fomite material (56). The current data show that Phi 6 infectivity persists longer in dried ATS (18°C, 20% RH, 3.04 g/m³ AH) than the Ebola Makona-WPGC07 strain persisted in blood under similar, but not exact, conditions (21°C, 40% RH, 7.35 g/m³ AH) (32). Overall, variations in log₁₀ reductions have been seen between species and strains of filoviruses (33) as previously mentioned. However, this comparison to the Makona variant in blood under similar conditions as used in this work suggests Phi 6 may be a conservative surrogate for Ebola at lower AH conditions (Table 1).

Differences in persistence for both Lake Victoria Marburgvirus (MARV) and Zaire Ebola virus (ZEBOV) were seen between metal (316 stainless steel) and plastic by Piercy et al (33). Both Lake Victoria MARV and ZEBOV suspended in guinea pig sera could not be recovered from metal surfaces at any time point regardless of the temperature and humidity. When placed on plastic, however, persistence improved with infectivity lasting up to 50 days at 4°C (33).

Non-porous surfaces appear to be a greater risk in transmission of EBOV in hospital settings than porous surfaces. Like EBOV and Coronavirus, Phi 6 did not persist as long on porous surfaces (TC and UC) as it did on non-porous surfaces (SS and PL) (Table 1,2). Cook et al.
(40) found the Makona variant of EBOV suspended in organic soil load was completely inactivated on cotton gowns within 24 h and persisted longer on steel carriers than PL (21.5°C, 30% RH, 5.69 g/m³ AH).

Sizun et al. (46) investigated the human coronavirus (HCV) 229E on cotton sponges and found that the virus declined rapidly as well, with no infective virus found after 12 h, and HCV OC43 was not infective after 1 h. Lai et al. (57) inoculated cotton gown fabric with SARS-CoV-1 and found survival for only 24 h when held at 20°C (RH not reported). No other controlled laboratory studies were found in the literature describing survival of other enveloped viruses on porous surfaces, though Abad et al. (58) demonstrated that persistence for some non-enveloped viruses such as Hepatitis A virus and bacteriophage B40-8 was lower if placed on cotton fabric than on hard surfaces, while other non-enveloped viruses (human rotavirus HRV, polio virus, enteric adenovirus) persisted as well or longer on cotton than on hard surfaces.

One limitation to this evaluation of Phi 6 bacteriophage as a suitable surrogate on porous materials is the difficulty in elucidating if there is a true lack of persistence or if the phage adhered to the fabric, making it difficult to elute. Adsorption to the curtains could potentially explain some of the decline in recoverable infective phage though the adsorption would most likely inhibit touch-transfer as well. Viral adsorption to fabrics is influenced by whether a fabric is tightly or loosely knit, the surface charge of the virus and of the fabric (59). These data inform public health investigators that enveloped viruses are likely to persist longer on surfaces in modern climate-controlled healthcare facilities than in tropical field stations. Additional precautions and disinfection strategies must be taken to prevent transmission when
treating infected patients. This work also provides additional evidence that Phi 6 may be considered a conservative surrogate for EBOV when conducting persistence investigations, in that it persisted longer. We also report that Phi6 may not be a suitable surrogate for coronaviruses in all environmental conditions, though there seems to be a wide range of persistence reported in the literature. The model for SARS-CoV-2 (60) predicted persistence of 4.4 days (99.99% inactivation), which is considerably more than our experimental finding of 73h for Phi 6. As reported by Aquino de Carvalo (24) when evaluating Phi 6 as a surrogate for persistence in water matrices, Phi 6 cannot not be considered a universal surrogate for persistence of all enveloped viruses on surfaces. Instead, multiple surrogate viruses should be considered if the virus of interest itself cannot be investigated.

**MATERIALS AND METHODS**

**Test materials**

Four test materials were chosen to simulate surfaces typically found within the U.S. healthcare environment and cut into 1 cm² coupons. The two non-porous surfaces were stainless steel (SS, 24-gauge, T-304: Stewart Stainless Supply, Suwanee, GA) and plastic (PL, PVC Acrylic Alloy, Kydex-T, 0.80 thickness, p-1 haircell texture, Kydex, LLC, Bloomsburg, PA). The two porous surfaces were polyester curtain fabric with (treated curtain, TC) and without (untreated curtain, UC) a zinc pyrithione treatment (ModoMed, Grand Rapids, MI). Non-porous (SS and PL) coupons were prepared by washing three times with dilute detergent Fisherbrand™ Versa-Clean™ (Unica Canada, Inc., Boucherville, Qc), rinsing with reverse osmosis water (3 times), and spraying with 70% ethanol. The SS coupons were sterilized by autoclaving at 121°C (30 psi) for
20 minutes. The PL, TC, and UC coupons were sterilized by UV (UVC wavelength of 254 nm) treatment for 30 minutes on each side.

*Phi 6 Propagation*

Bacteriophage Phi 6 and host *Pseudomonas syringae* were obtained from Laboratoire de Sylvain Moineau in Québec, Canada. Using prepared 18 h growth of *P. syringae* (HER1102) in 50 ml tryptic soy broth (TSB), Bacteriophage Phi 6 (HER102) were propagated from lyophilized stock by reconstituting with 1 ml of pre-warmed (37°C) TSB. Reconstituted Phi 6 (500 µl) was transferred into 50 ml fresh TSB with 100 µl of overnight host *P. syringae*, followed by gentle agitation using a vortex, and incubating with agitation (100-110 rpms) for 18 hours at 22°C. The Phi 6 lysate was then filter sterilized using a 0.22 µm syringe filter (PVDF, Millex-GV: Millipore, Burlington, MA) into a sterile 50 ml tube. The filtrate (Phi 6) was protected from light and stored in the dark at 4°C until experiments were performed. The Phi 6 stock titer was approximately 10⁸ plaque forming units per ml (PFUs/ml).

*Inoculation, and environmental exposure*

A 20% solution of Artificial Test Soil (ATS, Healthmark Industries Company Inc., Fraser, MI) was prepared in phage buffer (SM Buffer), according to the formulation in Cold Spring Harbor Protocols (61) [NaCl, MgSO₄·7H₂O, Tris-Cl (1 M, pH 7.5)], and used as a body fluid simulant, with ATS containing proteins (albumin), hemoglobin, carbohydrates, cellulose, and lipids. The stock Phi 6 was diluted in series to obtain a 10⁶ PFU/ml suspension in ATS. The coupons were inoculated with 10 µl of 10⁶ PFU/mL suspension in ATS, resulting in an inoculum of 10⁴ PFU/cm². Negative controls consisted of ATS (20%) without Phi 6 inoculated onto
coupons. The test coupons were placed in open petri dishes in triplicate, along with the negative coupons, in a Caron Model 6030 Environmental Chamber (Marietta, OH) at two controlled temperatures (T) and relative humidity (RH) levels; 26°C and 57% RH (AH = 14.4 g/m³) and 18°C and 20% RH (AH = 3.0 g/m³). Three inoculated coupons and a negative control were removed and processed immediately (T₀), and at designated time points until Phi 6 PFUs could no longer be detected (limit of detection from coupons was ≤2 PFU/cm²). Each experimental condition was repeated twice with triplicate coupons, for a total of 6 coupons at each time point. One exception is SS which had a total of 5 samples for each time point for the high AH (14.4 g/m³ AH) environmental condition.

Recovery

The phage(s) were dislodged from the coupons in 5 ml Phosphate Buffered Saline with Tween® 80 (PBST 0.02%) [0.01M PBS (7.2-7.4 pH) and Tween® 80 (0.02%)] by alternating between vortex and sonicating bath for 30 seconds each, repeating the rotation 3 times (62). The eluate was diluted in series in SM buffer. One mL of sample was plated with 500 µl of host P. syringae in tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, NJ), using a double agar overlay method (63). The overlay plates incubated for 18 – 24 h (22°C) before counting the PFUs. Coupon removal, processing and plating continued until no infective phage (as determined by PFU) were observed. PFUs/ml were calculated based on dilution factors, and log₁₀ transformed. Results were calculated in both relative humidity (RH) and absolute humidity (AH) to compare to other studies.

Statistical analysis
SAS v9.4 (Cary, NC) was used to create linear models that assessed the potential relationships between the mean log₁₀ change of virus concentration on SS, PL, TC and UC, at two environmental conditions (3.0 g/m³, 14.4 g/m³). For these analyses, the data points were used individually and not averaged. Least square methods were used to fit the model and determine the rate of log₁₀ reductions written as decay rate (log₁₀ reduction per day or hour) in Table 1 and 2 for this data; r² was used to assess goodness of fit of the model. Analysis of variance and the F statistic were used to test the differences between various materials (i.e., SS vs. PL, TC vs. UC, and porous vs. non-porous) under the same absolute humidity, where significance was set at a p-value < 0.05.

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FIG. 1 Model projection of low temperature and low humidity conditions (LTLH, AH=3.0 g/m³) on all surfaces, stainless steel (SS), plastic (PL), treated curtains (TC), and untreated curtains (UC). SS projected is represented by a dashed line and observed mean is a square. PL projected is represented by a solid line and observed mean is a triangle. The TC projected is represented by a dotted line and the observed mean is a circle. The UC projected persistence is represented by a dotted/dashed line and the observed mean is a diamond shape. Data points and error bars indicate mean and standard deviation of the projected data. Refer to Table 1 for $r^2$ values.
FIG. 2 Model projection of high temperature and high humidity conditions (HTHH, AH=14.4 g/m³ unit) on all surfaces, stainless steel (SS), plastic (PL), treated curtains (TC), and untreated curtains (UC). SS projected is represented by a dashed line and observed mean is a square. PL projected is represented by a solid line and observed mean is a triangle. The TC projected is represented by a dotted line and the observed mean is a circle. The UC projected persistence is represented by a dotted/dashed line and the average observed mean is a diamond shape. Data points and error bars indicate mean and standard deviation of the projected data. Refer to Table 1 for $r^2$ values.
TABLE 1 Persistence of bacteriophage Phi 6 compared to reports of Ebola Makona variant and human coronavirus on non-porous surfaces; stainless steel, aluminum, latex and plastic.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Surface</th>
<th>Matrix</th>
<th>RH (°C)</th>
<th>Decay Rate (r)</th>
<th>D-value (D_{\text{value}})</th>
<th>Time to No Detection (D_{\text{time}})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi 6</td>
<td>Steel</td>
<td>ATS</td>
<td>3.0</td>
<td>0.06 /d</td>
<td>0.88</td>
<td>18 d</td>
<td>102 d</td>
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<td></td>
<td></td>
<td>(18°C, 20%)</td>
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<td></td>
<td>Current data</td>
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<tr>
<td>Phi 6</td>
<td>Plastic</td>
<td>ATS</td>
<td>3.0</td>
<td>0.06 /d</td>
<td>0.96</td>
<td>14 d</td>
<td>78 d</td>
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<tr>
<td></td>
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<td>(18°C, 20%)</td>
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<td>Current data</td>
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<tr>
<td>Phi 6</td>
<td>Steel</td>
<td>ATS</td>
<td>14.4</td>
<td>1.42 /d</td>
<td>0.90</td>
<td>6 h</td>
<td>&gt; 3 d (77 h)</td>
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<td></td>
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<td>(26°C, 57%)</td>
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<td>Current data</td>
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<tr>
<td>Phi 6</td>
<td>Plastic</td>
<td>ATS</td>
<td>14.4</td>
<td>1.09 /d</td>
<td>0.91</td>
<td>6 h</td>
<td>&gt; 3 d (73 h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(26°C, 57%)</td>
<td></td>
<td></td>
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<td></td>
<td>Current data</td>
</tr>
<tr>
<td>Phi 6</td>
<td>Steel</td>
<td>Blood</td>
<td>2.7-11.3</td>
<td>N/A</td>
<td>N/A</td>
<td>77 -88 h(^a)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21.1-21.7°C, 15-59%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(56)</td>
</tr>
<tr>
<td>Phi 6</td>
<td>Steel</td>
<td>PBS</td>
<td>2.7-11.3</td>
<td>N/A</td>
<td>N/A</td>
<td>5 h</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21.1-21.7°C, 15-59%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(56)</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Organic soil load</td>
<td>5.7</td>
<td>0.22 /d</td>
<td>0.90</td>
<td>30 h</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21.5°C,30%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(42)</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Human Blood</td>
<td>7.4</td>
<td>0.69 /d</td>
<td>0.90</td>
<td>2 d</td>
<td>6 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21°C, 40%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(~4.5 log(_{10}))</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Human Blood</td>
<td>21.5</td>
<td>0.68 /d</td>
<td>0.87</td>
<td>2 d</td>
<td>7 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(27°C, 80%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(~5 log(_{10}))</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Human blood</td>
<td>3.3</td>
<td>0.79 /h</td>
<td>N/A</td>
<td>&lt; 12 h</td>
<td>&lt;3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22°C, 17%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(~3 log(_{10}))</td>
</tr>
<tr>
<td>Ebola</td>
<td>Plastic</td>
<td>Human blood</td>
<td>3.3</td>
<td>0.79 /h</td>
<td>N/A</td>
<td>&lt; 12 h</td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22°C, 17%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/A(^i)</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Human blood</td>
<td>8.0</td>
<td>0.63 /h</td>
<td>N/A</td>
<td>&lt; 12 h</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22°C, 41%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(~3 log(_{10}))</td>
</tr>
<tr>
<td>Ebola</td>
<td>Plastic</td>
<td>Human blood</td>
<td>8.0</td>
<td>0.63 /h</td>
<td>N/A</td>
<td>&lt; 12 h</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22°C, 41%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/A(^i)</td>
</tr>
<tr>
<td>Ebola</td>
<td>Steel</td>
<td>Human blood</td>
<td>25.8</td>
<td>0.29 /h</td>
<td>N/A</td>
<td>~72 h</td>
<td>10 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28°C, 90%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(~3 log(_{10}))</td>
</tr>
<tr>
<td>Virus</td>
<td>Material</td>
<td>Contact Medium</td>
<td>Initial Log10 Decay Rate</td>
<td>Rate of Decay</td>
<td>Days to 99.99% Reduction</td>
<td>Conditions Not Tested</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------------------------</td>
<td>---------------</td>
<td>--------------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td>Ebola</td>
<td>Plastic</td>
<td>Artificial Saliva</td>
<td>1.4 - 4.0 (26°C, 57%)</td>
<td>0.29/h N/A</td>
<td>4.4 d for 99.99% reduction</td>
<td>(~2.5 log10)</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Plastic and Steel</td>
<td>N/A</td>
<td>7.4 - 8.4 (21-23°C, 40%)</td>
<td>8 - 24 h N/A</td>
<td>3.4 d (~3.7 log10a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Plastic</td>
<td>Culture medium</td>
<td>12.7 (22°C, 65%)</td>
<td>N/A</td>
<td>7 d (~5.8 log10a)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Steel</td>
<td>Culture medium</td>
<td>12.7 (22°C, 65%)</td>
<td>N/A</td>
<td>7 d (~5.8 log10a)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td>Plastic and Steel</td>
<td>N/A</td>
<td>7.4 - 8.4 (21-23°C, 40%)</td>
<td>8 - 24 h N/A</td>
<td>3.4 d (~3.4 log10a)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td>Plastic</td>
<td>Culture medium</td>
<td>7.8 - 11.9 (22-25°C, 40 - 50%)</td>
<td>N/A</td>
<td>28 d (~5 log10a)</td>
<td>(64)</td>
<td></td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Plastic and Steel</td>
<td>Culture medium</td>
<td>6.9 (20°C, 40 RH)</td>
<td>8 - 10 h N/A</td>
<td>3 d (~5 log10a)</td>
<td>(39)</td>
<td></td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Plastic and Steel</td>
<td>Culture medium</td>
<td>26.1 (30°C 80%RH)</td>
<td>N/A</td>
<td>1 d (~5 log10a)</td>
<td>(39)</td>
<td></td>
</tr>
<tr>
<td>HCV OC43</td>
<td>Latex</td>
<td>Culture medium</td>
<td>10.1 - 13.8 (21°C, 55-75%)</td>
<td>&lt;1 h N/A</td>
<td>1 h (~3 log10a)</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>HCV 229E</td>
<td>Aluminum and Latex</td>
<td>Culture medium</td>
<td>10.1 - 13.8 (21°C, 55-75%)</td>
<td>&lt;1 h N/A</td>
<td>1 h (~3 log10a)</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>HCV OC43</td>
<td>Aluminum</td>
<td>Culture medium</td>
<td>10.1 - 13.8 (21°C, 55-75%)</td>
<td>~4-5 h N/A</td>
<td>12 h (~3 log10a)</td>
<td>(46)</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
1. Decay rates for current data based upon model predictions graphed in Figure 1 and 2 expressed in log10. Other decay rates taken from literature.
2. Artificial Test Soil
3. N/A = no data
4. Medians of two experiments
5. Conditions not actually tested, but estimated from model predictions.
6. Conditions not actually tested, but estimated from model predictions.
TABLE 2 Persistence of bacteriophage Phi 6 within an Artificial Test Soil (ATS) matrix on porous hospital curtains, one treated with zinc pyrithione (TC) and one untreated (UT). Human Coronavirus studies on other porous surfaces are presented for comparison. Limited comparison data from the literature for three coronaviruses are also presented.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Surface</th>
<th>Matrix</th>
<th>AH g/m³ (°C, RH)</th>
<th>Decay Rate¹</th>
<th>D-value</th>
<th>Time to No Detection (log₁₀ reduction @ last sampling point detected)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi 6</td>
<td>TC</td>
<td>ATS²</td>
<td>3.0 (18°C, 20%)</td>
<td>0.11/d</td>
<td>0.72</td>
<td>30 min (5.40 log₁₀ @ 35 d)</td>
<td>Current Data</td>
</tr>
<tr>
<td>Phi 6</td>
<td>UC</td>
<td>ATS²</td>
<td>3.0 (18°C, 20%)</td>
<td>0.10/d</td>
<td>0.71</td>
<td>30 min (5.27 log₁₀ @ 35 d)</td>
<td>Current Data</td>
</tr>
<tr>
<td>Phi 6</td>
<td>TC</td>
<td>ATS²</td>
<td>14.4 (26°C, 57%)</td>
<td>0.65/h</td>
<td>0.71</td>
<td>&lt;30 min (2 log₁₀)</td>
<td>Current Data</td>
</tr>
<tr>
<td>Phi 6</td>
<td>UC</td>
<td>ATS²</td>
<td>14.4 (26°C, 57%)</td>
<td>0.71/h</td>
<td>0.49</td>
<td>&lt;30 min (3 log₁₀)</td>
<td>Current Data</td>
</tr>
<tr>
<td>HCoV 229</td>
<td>Cotton Gauze</td>
<td>Growth medium (21°C, 55-70%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>12 h (3 log₁₀)</td>
<td>(46)</td>
</tr>
<tr>
<td>HCoV OC43</td>
<td>Cotton Gauze</td>
<td>Growth medium (21°C, 55-70%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1 h (3 log₁₀)</td>
<td>(46)</td>
</tr>
<tr>
<td>SARS CoV-1</td>
<td>Cotton Gown</td>
<td>Growth medium</td>
<td>20°C, RH N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1 d (6 log₁₀)</td>
</tr>
</tbody>
</table>

¹ Decay rates for current data based upon model predictions graphed in Figure 1 and 2 expressed in log₁₀.
² Artificial Test Soil
³ N/A = no data

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References


