# Evaluation of environmental conditions as a decontamination approach for SARS-CoV-2 when applied to common library, archive and museum-related materials

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#### Abstract

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Aims: The purpose of this study was to evaluate the effects of ambient or altered environmental conditions on the inactivation of SARS-CoV-2 applied to materials common in libraries, archives and museums.

Methods and Results: Porous and non-porous materials (e.g. paper, plastic protective book cover) were inoculated with approximately  $1 \times 10^5$  TCID<sub>50</sub> SARS CoV-2 (USA-WA1/2020), dried, placed within test chamber in either a stacked or unstacked configuration, and exposed to environmental conditions ranging from 4 to 29°C at  $40 \pm 10\%$  relative humidity. The amount of infectious SARS-CoV-2 was then assessed at various timepoints from 0 to 10 days. Ambient conditions resulted in varying inactivation rates per material type. Virus inactivation rate decreased when materials were stacked or at colder temperatures. Virus inactivation rate increased when materials were unstacked or at warmer temperatures.

Conclusions: SARS-CoV-2 at ambient conditions resulted in the inactivation of virus below limit of quantitation (LOQ) for all materials by Day 8. Warmer temperatures, for a subset of materials, increased SARS-CoV-2 inactivation, and all were <LOQ by Day 3. Significance and Impact of the Study: These results provide information for the library, archives and museum community regarding the inactivation of SARS-CoV-2, showing that inactivation is possible using prescribed environmental conditions and is a potential method of decontamination for items not compatible with common liquid disinfectants.

#### **KEYWORDS**

archives, books, COVID-19, decontamination, inactivation, libraries, museums, paper, persistence, plastic, SARS-CoV-2

## **INTRODUCTION**

The World Health Organization declared severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

a global pandemic on 11 March 2020 (World Health Organization, 2020). The rapid onset of the coronavirus disease 2019 (COVID-19) pandemic meant that industries that serve the public had to make operational decisions without

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clear evidence regarding the mode of transmission, viral shedding, infectious dose, the persistence or decontamination of this novel pathogen in the environment, and many other characteristics of the virus. Among these industries are cultural institutions (e.g. libraries, archives, museums) that provide information, educational resources, collection items (e.g. books, DVDs) to borrow or visit, and spaces for people to gather and learn. There are approximately 2.6 million libraries across the world; in the United States, there are approximately 130,000 libraries, including more than 17,000 public library outlets. These libraries hold billions of collection items; in 2019, there were nearly 2.2 billion circulations of items from U.S. public library collections alone, half of which were books. In 2019, more than 174 million registered users visited public libraries over 1.2 billion times; and 125 million people attended 5.9 million programmes at a local library (Pelczar et al., 2021). There are also approximately 35,000 museums in the United States; these institutions preserve, protect and share more than a billion objects (Heritage Preservation, 2005). There are approximately 850 million visits each year to U.S. museums, including approximately 55 million visits each year from students in school groups (IMLS, 2018).

When the pandemic was declared, directors and staff at these cultural institutions sought science-based information about how to reduce the risk of transmitting the virus to staff, volunteers and visitors as they adapted operations and policies for their respective spaces. The need for science-based information specific to the distinct services of libraries, archives and museums (LAMs) led to a partnership between the Institute of Museum and Library Services (IMLS), OCLC and Battelle to study the active lifespan of SARS-CoV-2 on materials frequently handled by staff and the public from those institutions.

Since then, studies of similar types of pathogens and SARS-CoV-2 have shown the primary route of disease transmission is via aerosol or direct droplet contact (Chia et al., 2020; Greenhalgh et al., 2021; Guo et al., 2020; Zhang et al., 2020); in some cases, however, SARS-CoV-2 has also been shown to remain infectious on surfaces for multiple days (Chin et al., 2020; Van Doremalen et al., 2020). As a result, virus may be spread from person to person by touching contaminated surfaces, which may be a secondary route of transmission. LAMs manage a vast quantity of physical collection items that are frequently handled by both staff and visitors, and, in the case of libraries, collection items are brought into homes, vehicles, and public places for weeks and then returned to the library. For these reasons, there was justified concern about the potential for surface-based transmission for people utilizing these items.

Many industries serving public needs (e.g. food, health) have been negatively impacted by the pandemic and, in an effort to safely remain open for business, have implemented

several mitigation strategies such as social distancing, masking and improved ventilation, as well as various surface disinfection methods (Ebrahim et al., 2020; Ferioli et al., 2020; Morawska et al., 2020; Rader et al., 2021). Several decontamination approaches (i.e. vapour phase hydrogen peroxide, formaldehyde) have been shown to be effective for the inactivation of biological Select Agents (Calfee & Wendling, 2015; Richter et al., 2018; Rogers et al., 2005; Rogers et al., 2007; Rogers & Choi, 2008; Wood et al., 2016), as well as SARS-CoV-2 (Chin et al., 2020; Kratzel et al., 2020; Raeiszadeh & Adeli, 2020; Ratnesar-Shumate et al., 2020). However, the logistics of scaling these technologies can be challenging, particularly in the case of LAMs, where large outdoor areas, operations or buildings and its frequently handled collection items are affected. These challenges often involve fumigants or liquids that can be dangerous to human health, harmful to the environment or deleterious to the materials being decontaminated. Many of the items identified for this study were not suitable for routine liquid disinfection techniques, such as hard copies of books, archival materials or fabrics. Consequently, an alternate approach was required to ensure the virus would not be circulated back into the collections/institutions and, subsequently, transmitted to staff or other patrons. Access to physical materials are core functions of nearly all types of LAM operations, underscoring the importance of this type of research.

The survival or persistence of biological organisms in the environment has been previously studied and is largely influenced by the climate and the materials with which these biological organisms are in contact (Biryukov et al., 2020; Calfee & Wendling, 2012; Casanova et al., 2010; Chin et al., 2020; Kampf et al., 2020; Richter et al., 2019; Rogers et al., 2016; Wood et al., 2015; Wood et al., 2018). Environmental factors that may impact the viability of bacteria and the infectivity of viruses in the environment include temperature, humidity, ultraviolet (UV) radiation and desiccation (Sinclair et al., 2008). Previous studies have examined the persistence of viral Select Agents, including severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (Bedrosian et al., 2020; Berendt & Dorsey, 1971; Brown et al., 2014; Graiver et al., 2009; Lai et al., 2005; Liu et al., 2021; Pastorino et al., 2020; Pyankov et al., 2012; Riddell et al., 2020; Sagripanti et al., 2010; Verreault et al., 2013; Yamamoto et al., 2010), and have shown the ability of these agents to persist on various transferable surfaces (i.e. fomites) for days to months. Many of these studies, however, did not examine the effects when applied to materials commonly found in the LAMs settings.

The purpose of this study was to evaluate the persistence of SARS-CoV-2 under ambient (indoor, climatecontrolled) environmental conditions, over prescribed durations, on materials commonly found in LAMs. Due to the complicated logistics required to maintain thousands to millions of books and other items, this study focused on the high-touch aspect of commonly circulated materials such as book covers and DVD cases. Because these items are also typically held in shelves, either 'stacked' or positioned 'unstacked' on display cases, it was necessary to evaluate persistence of the virus in configurations that replicate the most common real-world operational conditions. In addition, since LAMs operate under a broad range of climates to preserve and make items accessible, alternate conditions (warm and cold) were evaluated. Warm temperature conditions were also evaluated to determine whether these altered conditions increase the rate of virus attenuation and provide a rapid means of decontamination for these materials. This study presents data on the persistence of SARS-CoV-2 under ambient laboratory conditions and altered environmental conditions, both in terms of total recovery and duration. The study sought to identify when the virus reached an infectivity level that measured below the limit of quantitation (LOQ) of the assay used.

## MATERIALS AND METHODS

## Test organism

SARS-CoV-2 strain USA-WA1/2020 was obtained from BEI Resources and propagated in Vero (African green monkey kidney) clone E6 cells (BEI Resources product No. NR-596). The cells were incubated at 37°C with 5% carbon dioxide  $(CO_2)$  in complete cell culture media (Eagle's Minimum Essential Medium, Corning Cat. No. 10-009-CV) supplemented with 10% foetal bovine serum (Gibco Cat. No. 10082147) and penicillin-streptomycin (PS; Gibco Cat. No. 15140122) until approximately 90% cell confluency was achieved. Viral propagation was performed using a roller bottle method (Glasbrenner et al., 2021) using 2 ml of SARS-CoV-2 stock at a multiplicity of infection (MOI) of 0.001, along with 5 ml of inoculation media (Minimum Essential Medium, Corning Cat. No. 10-010-CV, containing 5% FBS and PS) and allowed to infect for 1 h at 37°C with 5% CO<sub>2</sub>. After infection, 25 ml of complete MEM (2% FBS, PS) was added, and incubation continued for 36-48 h at 37°C with 5%  $CO_2$  and 5 revolutions per minute (rpm). Once cytopathic effect (CPE) was observed throughout the flask, cells were removed by trypsinization (0.25% Trypsin-EDTA, Gibco 25,200-056). Harvested cells were vortexed for 2 min at maximum speed with a ratio of 1:7 sterile glass beads (Sigma-Aldrich, Cat. No. CLS72685) to cells and then centrifuged at 800g for 5 min at 4°C to remove any remaining cellular debris. The resulting supernatant was frozen at -80°C in single-use vials.

# **Test materials**

In all, 25 material surfaces common to the LAMs settings were used for testing as outlined in Table 1. These test coupons (1.9 centimetre  $[cm] \times 7.5 cm$ ) were cut from a larger piece of material stock and were used as received from the supplier; the test coupons were not sterilized prior to use. Visual inspection of the physical integrity of the test coupons was performed prior to and after testing to assess any damage or change to the coupons. Selected materials (hard- and softback book covers, plain paper pages, plastic protective cover and DVD case) were tested in a stacked and, in some cases, unstacked configurations. For the stacked configuration, the materials were inoculated with the SARS-CoV-2, allowed to dry, and then the inoculated surface side was inverted and placed in contact with a like material to replicate routine storage conditions. The unstacked configuration consisted of the inoculation of the materials followed by drying, where the inoculated surface remained upright and open to the surrounding test chamber environment.

## Sample processing and data collection

All work with SARS-CoV-2 was conducted in a Biosafety Level 3 (BSL-3) laboratory. The test organism was prepared in a simulated saliva matrix by concentrating virus stock material in a 100 K MWCO protein concentrator (Pierce Protein Concentrator PES, Thermo Scientific Cat. No. PI88533) to approximately 0.2 ml then adding the appropriate volume of synthetic saliva. Synthetic saliva was prepared according to ASTM E2721 using porcine as the mucin source (Heimbuch et al., 2011). Coupons were laid flat in a Class II Biological Safety Cabinet (BSC) and inoculated with approximately  $1 \times 10^5$  median tissue culture infectious dose (TCID<sub>50</sub>) per coupon. A 100-microliter ( $\mu$ l) aliquot of test organism (approximately  $1 \times 10^6 \text{ TCID}_{50}$  $ml^{-1}$ ) was dispensed as 10 droplets (10 µl per droplet) across the surface of the test coupons. For each type of material, five coupons were used to assess persistence of the organism at each combination of environmental condition and timepoint tested. All material coupons were allowed to dry for 1 h in the BSC under ambient conditions (approximately 22°C and 40% relative humidity [RH]) before testing. Additionally, one coupon of each material was used as a negative control (not inoculated) and were included for each timepoint tested. The blank coupons controlled for potential cross-contamination during testing as well as for sterility and potential cytotoxic effects from the test coupons.

Coupons inoculated with SARS-CoV-2 were exposed to various combinations of temperature and humidity,

	Material type	Description of material	Source of material
1	Hardback book cover	Buckram cloth	Columbus Metropolitan Library
2	Softback book cover	Coated paper	
3	Plain paper pages	Uncoated, plain paper	
4	Plastic protective cover	Biaxially oriented polyester film	
5	DVD case	Polypropylene	
6	Children's board book	Coated board book	
7	DVD	Polycarbonate	
8	Storage bag	Low-density polyethylene	
9	Storage container	High-density polyethylene	
10	Glossy page	Found in a coffee table book	
11	Magazine page	Glossy magazine page	
12	Archival folder	10 pt. folder stock with a 3% calcium carbonate buffer (pH 8.5)	National Archives and Records Administration
13	Plexiglass	Acrylic	
14	Expanded polyethylene foam	Polyethylene foam (1" thick)	
15	Braille page	Braille pages	Library of Congress
16	USB cassette	Acrylonitrile butadiene styrene	
17	Powder-coated steel	Powder coated book end	
18	Nylon webbing	Nylon weave	American Museum of Natural History
19	Leather book cover	Leather (circa 1861)	Private donation
20	Marble	Danby marble	National Park Service
21	Laminate	Laminate with particle board backing	Metropolitan New York Library Council
22	Synthetic leather	Expanded polyvinyl chloride	Commercially acquired
23	Polyolefin fabric	100% polyolefin	
24	Glass	Plain glass (no coating)	
25	Brass	260 series brass	

storage configuration (i.e. stacked, unstacked) for up to 10 days as outlined in Table 2. At each timepoint tested, SARS-CoV-2 viability was assessed. After inoculation and the initial drying period, the coupons were placed into airtight test chambers (Lock and Lock, HPL838P) that were pre-conditioned to the prescribed environmental parameters. The test chambers were placed into an incubator, devoid of light and maintained at 4, 22 or  $28 \pm 2^{\circ}$ C (Innova 4230, New Brunswick Scientific) for controlled temperature exposure. Control of RH conditions (approximately 40%) were accomplished by adding a container of saturated magnesium chloride  $(MgCl_2)$  to the bottom of the test chamber (American Society for Testing and Materials, 2007). A data logger (Onset Hobo MX1101) was placed at the bottom of the test chamber to monitor and record both temperature and RH every minute for the duration of testing. At time 0 (approximately 1 h following inoculation when the virus appears

dried) and at the end of each timepoint, samples were collected and extracted by placing each coupon in a conical tube that contained 10 ml of inoculation media. All vials were agitated on their sides at room temperature on an orbital shaker (Thermo Scientific Solaris) for 15 min at 200 rpm. Extracts were transferred to a concentrator (Pierce Protein Concentrator PES, Thermo Scientific Cat. No. PI88533) and centrifuged at 3000g until the 10 ml starting volume was concentrated to approximately 0.5 ml. Approximately 10 ml of fresh inoculation media was added to the concentrated sample (i.e. retentates) for the purpose of washing and removing any potentially cytotoxic chemicals extracted from the materials themselves (i.e. a buffer exchange). The concentrator was centrifuged again and concentrated to approximately 0.5 ml. Media was added to equilibrate all washed extracts to approximately 2 ml. Washed extracts were passed through a 0.2-micron PES syringe filter (Corning

#### TABLE 2 Overview of test matrix and environmental conditions

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Test number	Materials	Test configuration	Timepoints (days)	Actual environmental conditions
1	Hardback book cover	Unstacked	0, 1, 3, 4	23.0 ± 0.6°C 41.2 ± 4.7%
	Softback book cover	Unstacked		RH
	Plain paper pages	Unstacked		
	Plastic protective cover	Unstacked		
	DVD case	Unstacked		
2	Children's board book	Unstacked	0, 1, 2, 3, 4	$21.8 \pm 0.5^{\circ}$ C $41.8 \pm 1.9\%$
	Archival folder	Unstacked		RH
	Braille page	Unstacked		
	Glossy page	Unstacked		
	Magazine page	Unstacked		
3	Talking book USB cassette	Unstacked	0, 2, 3, 4, 5	$21.9 \pm 0.6^{\circ}$ C $37.4 \pm 0.9\%$
	DVD	Unstacked		RH
	Storage bag	Unstacked		
	Storage container	Unstacked		
	Plexiglass	Unstacked		
4	Hardback book cover	Stacked	0, 2, 3, 4, 6	$21.8 \pm 0.3^{\circ}$ C $38.6 \pm 1.8\%$
	Softback book cover	Stacked		RH
	Plastic protective cover	Stacked		
	DVD case	Stacked		
	Expanded polyethylene foam	Unstacked		
5	Leather book cover	Unstacked	0, 2, 4, 6, 8	$21.7 \pm 0.1^{\circ}$ C $35.3 \pm 1.8\%$
	Synthetic leather	Unstacked		RH
	Polyolefin fabric	Unstacked		
	Cotton fabric	Unstacked		
	Nylon webbing	Unstacked		
6	Glass	Unstacked	0, 2, 4, 6, 8	$21.7 \pm 0.1^{\circ}$ C $36.6 \pm 0.8\%$
	Marble	Unstacked		RH
	Laminate	Unstacked		
	Powder-coated steel	Unstacked		
	Brass	Unstacked		
7	Expanded polyethylene foam	Unstacked	0, 2, 3, 4, 6, 8, 9, 10	2.4 ± 1.3°C 34.5 ± 3.6% RH
	Hardback book cover	Stacked		
	Softback book cover	Stacked		
	Plastic protective cover	Stacked		
8	Expanded polyethylene foam	Unstacked	0, 2, 3, 4, 6, 8, 9, 10	$28.6 \pm 0.3^{\circ}$ C $32.6 \pm 0.5\%$ RH
	Hardback book cover	Stacked		
	Softback book cover	Stacked		
	Plastic protective cover	Stacked		

Cat. No. 431229) to remove any coupon debris released during the extraction process or potential endogenous contaminants that may interfere with the assay. Each material extract was assessed for infectious SARS-CoV-2 using an endpoint dilution assay to determine the  $TCID_{50}$ . Additionally, each extract was

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evaluated for potential cytotoxic effects from the test materials. Serial dilutions (fivefold) were completed in inoculation media and plated onto 80%-90% confluent Vero E6 monolayers, followed by evaluations for CPE 72-120 h post-infection. Quantification of infectious SARS-CoV-2 was determined via the Spearman-Karber method (Hamilton et al., 1977). The LOQ for the SARS-CoV-2 TCID<sub>50</sub> assay was 13.1 TCID<sub>50</sub> ml<sup>-1</sup> (1.12 log<sub>10</sub>  $TCID_{50}$ ). Once below this threshold, the assay can no longer assign a quantitative value output; however, a qualitative assessment of the presence of infection can be observed through manual microscopic examination. Therefore, any values below LOQ, but positive for presence of virus, are assigned an arbitrary value of 10 (indicating positive) to allow it to be resolved from 0 (indicating negative), presence of viral infection in the host cells. An average is calculated for the values assigned to the five test coupons for each material per timepoint.

## RESULTS

A total of eight tests were conducted to assess the survival of SARS-CoV-2 on 25 different test material surfaces. The mean amount of virus applied to each of these test materials across all tests was  $4.98 \pm 0.35 \log_{10} \text{TCID}_{50}$ . The mean recovery of SARS-CoV-2 after the 1-hour dry time (time-point 0 or T0) across all tests was  $3.09 \pm 0.74 \log_{10} \text{TCID}_{50}$ .

except for a notable outlier of brass that resulted in only  $0.78 \log_{10} \text{TCID}_{50}$  recovery. This resulted in an average 1.9 log reduction (LR) after the SARS-CoV-2 was allowed to dry for 1 h on the material surfaces under ambient environmental conditions.

#### Ambient temperature

Figure 1 shows the duration required for inactivation of SARS-CoV-2 below the LOQ of the  $TCID_{50}$  assay under ambient and altered temperature conditions, which for the 2 ml extract volume was 26.2  $TCID_{50}$ . In all, 24 materials resulted in levels below LOQ after 4 days of exposure and the last remaining material (synthetic leather) persisted to 8 days. While statistical analysis was not conducted, the survival of SARS-CoV-2 virus and the porosity of the material type did not result in any obvious paired effect that has been previously observed for other similar environmental persistence evaluations (e.g. porous materials result in slower reduction in viability while non-porous materials result in faster reduction; Richter et al., 2019).

## Material storage configuration

Five test materials (DVD case, hardback book cover, plain paper pages, plastic book covering, softback book



**FIGURE 1** Duration (days) required to achieve inactivation of SARS-CoV-2 below LOQ. ● indicates Ambient; ⊕ indicates Ambient Stacked; ▲ indicates cold; ▲ indicates Cold Stacked; ■ indicates Warm; ⊞ indicates Warm Stacked; (1) Adjusted LOQ of 65.5 and 327.5 TCID50 for polyolefin fabric and nylon webbing, respectively

cover) were evaluated at ambient temperature conditions in a stacked configuration, to reflect routine storage conditions of the materials common in library facilities. Except for plain paper pages, all materials were also evaluated in an unstacked configuration to allow for direct comparison against the stacked configuration. These items were tested from 0 to 6 days. Overall, items stored in a stacked configuration resulted in greater persistence of the SARS-CoV-2 virus as compared to the unstacked materials (Figure 2). All materials tested in the unstacked configuration resulted in detection levels below LOQ after only 1 day of exposure. However, when stacked, these same materials required 3-4 days to reach detection levels below LOQ. The hardback book cover was the only exception, which continued to result in recovery greater than LOQ on the final day of testing (day 6).

#### **Temperature dependence**

The ability of raising (28°C) or lowering (4°C) the storage temperature was also evaluated on four test materials (hardback book cover, softback book cover, plastic protective cover and expanded polyethylene foam, a commonly used material in museum settings for storage, shipping and displays) that had also been tested under ambient conditions (22°C). All materials except the expanded polyethylene foam were evaluated in the stacked configuration. A sample of each material was evaluated at each of the three environmental conditions from 0 to 10 days (Figure 3).

The cold temperature test condition  $(4 \pm 2^{\circ}C)$ , which was an 18° delta in temperature from ambient conditions, resulted in less attenuation on all surfaces when compared to both ambient and warm test conditions. Reduction in SARS-CoV-2 infectivity, due to the temperature reduction, for all materials was less than or equal to 0.6 LR or lower over the course of the 10 days of exposure, with the exception of the hardback book cover. The hard book cover followed a similar trend until day 9, when detectable levels fell below LOQ.

Warm temperature testing  $(28 \pm 2^{\circ}C)$ , which was only a 6° delta from ambient conditions, resulted in slightly faster attenuation rates when compared to ambient conditions. At the elevated temperature of 28°C, viral infectivity fell below the LOQ threshold for all four materials after 3 days of exposure. By comparison, exposure at ambient conditions resulted in longer exposure durations for plastic protective cover, expanded



**FIGURE 2** Comparison of storage configuration  $(22\pm 2^{\circ}C \text{ and } 40\pm 10\% \text{ RH})$  on the inactivation of SARS-CoV-2 for stacked and unstacked materials  $\pm$  95% confidence interval. (•) indicates Unstacked; (•) indicates Stacked (---) indicates LOQ



FIGURE 3 Comparison of storage temperature on the inactivation of SARS-CoV-2 ± 95% confidence interval. (•) indicates Ambient; (▲) indicates Cold; (■) indicates Warm; (---) indicates LOQ

polyethylene foam and hardback book cover of 4 and 6 days, respectively. No change in exposure duration was observed for softback book cover as both ambient and warm test conditions resulted in viral infectivity below LOQ by day 3.

# Cytotoxicity

All materials were tested as received (i.e. no cleaning or sterilization of the materials prior to testing); therefore, the potential for endogenous contaminants was mitigated by filtering each sample as described above and verified using negative controls for each material type throughout testing. No evidence of endogenous contamination was observed for any of the materials during testing. The negative controls also allowed for evaluation of potentially cytotoxic chemicals that could be released from the materials during the coupon extraction process. Four materials tested (cotton fabric, polyolefin fabric, nylon webbing and new leather) resulted in observed cytotoxic effects. Two materials (polyolefin fabric and nylon webbing) were partially quantifiable since the CPE was able to be diluted out (fivefold dilutions) as part of the viability assay. The

dilutions resulting in cytotoxic effect were excluded from evaluation, resulting in adjusted LOQ values of 65.5 and 327.5  $\mathrm{TCID}_{50}\ \mathrm{ml}^{-1}$  for polyolefin fabric and nylon webbing, respectively. The other two materials (cotton fabric and 'new' leather) resulted in cytotoxicity that was not readily diluted, and as a result, these materials were excluded from testing. It is worth noting that the first leather type evaluated was 'old' book binding leather (circa 1861), which resulted in no cytotoxic effects and was used for testing. Further evaluations using leather were intended, but due to limited availability of the original material, a more widely available 'new' leather was selected, which resulted in cytotoxicity and was excluded testing.

## DISCUSSION

When evaluating decontamination strategies for organizations such as LAMs to address potential surface contamination with SARS-CoV-2 virus, both the type and scale of materials used by these organizations required the evaluation of non-traditional decontamination methods. While fomite contamination is not considered the primary route of exposure for SARS-CoV-2, certain

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populations such as persons with disabilities or children may be at increased risk from this mode of transmission (Gleason et al., 2021; Kraay et al., 2021). The use of environmental conditions as an approach to decontaminate or inactivate other biological organisms has been previously studied and offers advantages of safe deployment as well as rapid scalability. This approach requires no special equipment beyond HVAC building controls to maintain targeted environmental conditions. The downside to this approach is the increased time or the increased operating cost required to maintain elevated temperatures to obtain the desired result, compared to the rapid effects of using typical liquid or fumigant disinfection techniques.

All aspects of this study were intended to approximate real-world conditions. To achieve these conditions, pre-circulated (used) materials from LAM institutions were used (when possible), and the evaluation of typical storage or display condition configurations (i.e. stacked vs unstacked) was included. The virus was resuspended in a synthetic saliva formulation to replicate direct droplet inoculation that could result from a cough or sneeze, and this mixture was used for inoculation of the test material surface. The amount of virus applied to each test material was intended to represent a realistic, albeit worst-case contamination scenario of direct droplet exposure (approximately  $4.98 \pm 0.35 \log_{10} \text{TCID}_{50}$ ). The application of this high titre inoculum was recently substantiated by data showing high viral loads (>7  $\log_{10}$  $TCID_{50}$  ml<sup>-1</sup>) of the SARS-CoV-2 Delta variant in the nasopharyngeal swabs of vaccinated healthcare workers (Chau et al. 2021).

The results of this study have shown that the SARS-CoV-2 virus will naturally attenuate within 4-8 days when exposed to ambient temperature and humidity conditions below the LOQ of the assay. This is applicable to many surface types (not suitable for routine liquid disinfection methods) relevant to the LAMs communities. This study has also confirmed that storage under warm conditions will increase the rate of natural attenuation and reduce the time required to achieve LOQ by 0-3 days based on material type, and conversely, under cold storage conditions, the attenuation rate steeply declines. These findings are consistent with similar research efforts where the ability to achieve 6 LR or greater for Venezuelan Equine Encephalitic virus on both porous and non-porous materials was achieved in 6-12 h as compared to control samples held at ambient temperatures showing minimal LR after 96 h. (Richter et al., 2019). More recently, the half-life of SARS-CoV-2 virus on non-porous fomites stored at 24°C ranged from 6.3 to 18.6 h; however, by increasing the temperature to 35°C, the half-life was reduced to 1.0 to 8.9 (Biryukov

et al., 2020). Additionally, the way items are stored (stacked vs. unstacked) was found to impact attenuation rates such that when like materials are stacked together (in the absence of light), the persistence of the virus increased by 2-3 days as compared to items left unstacked and exposed to the surrounding environment. This increased viral stability may be the result of reduced airflow resulting from this storage configuration. Further evaluation, increasing airflow across test materials, may help to inform this hypothesis should the opposite result be achieved. These results provide much-needed scientific findings that can be used to inform how LAMs manage collections during the pandemic. Of note, this study suggests that realistic, lowcost procedures can be implemented to help reduce the risk of spreading SARS-CoV-2 via fomites among staff and patrons. This study did not examine the effect of lower concentrations of inoculum or storage temperatures above 28°C, both of which may result in shorter durations to achieve LOQ which should be considered for future evaluations.

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#### **CONFLICT OF INTEREST**

No conflict of interest declared.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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