REALM REOpening Archives, Libraries, and Museums PROJECT

Test 4: Natural attenuation as a decontamination approach for SARS-CoV-2 on stacked library materials and expanded polyethylene foam

In response to the COVID-19 pandemic, the Institute of Museum and Library Services (IMLS) and OCLC are working in partnership with Battelle to distribute science-based information designed to help reduce the risk of transmission of COVID-19 to staff and visitors who are engaging in the delivery or use of archival, library, and museum services. This REopening Archives, Libraries, and Museums (REALM) project is studying how long the SARS CoV-2 virus (the virus that causes COVID-19) survives on common materials and methods to mitigate exposure.

As part of the project's Phase 1 and 2 research, Battelle has conducted four natural attenuation studies to provide information on how long some materials commonly handled in archives, libraries, and museums would need to be considered for quarantine prior to being put back into use. The results of Tests 1 through 3 were released on June 22, July 20, and August 18, 2020, respectively; Test 4 began on July 31, 2020.

Each study has been conducted by applying the virulent SARS-CoV-2 virus on five materials held at standard room temperature (68°F to 75°F) and relative humidity conditions (30 to 50 percent). The materials in Test 4 included four items previously examined in Test 1, but for this test they were placed in a stacked configuration. This configuration resembles the common practice used by libraries when handling book returns. In addition, expanded polyethylene foam, a commonly used material in museum settings for storing and shipping displays, was placed in an unstacked configuration. All items tested are listed in Table 1 below. Items were provided by the Columbus Metropolitan Library* and the National Archives and Records Administration.** Samples from each item were inoculated, allowed to dry, and were then inverted and placed on top of a larger piece of the same material type (to create stacking effect). A small section of book pages was used to weigh down the softcover book cover and the plastic protective cover to create a flat surface-to-surface contact. The items were then examined two, three, four, and six days after the initial T0 evaluation. Day six was the final timepoint tested.

Table 1. Test 4 items examined.

Item	Material type	Use
Hardcover book cover*	Buckram cloth	Hardcover book covering
Softcover book cover*	Coated paper	Trade paperback cover
Plastic protective cover*	Biaxially oriented polyester film	Protective layer for hardcover books
DVD case*	Polypropylene	Storage of DVD and CD media
Expanded polyethylene foam**	1-in. polyethylene foam	Storage and shipping

Results show that after six days of quarantine the SARS-CoV-2 virus was still detected on all five materials tested. When compared to Test 1, which resulted in nondetectable virus after three days on an unstacked hardcover book, softcover book, plastic protective cover, and DVD case, the results of Test 4 highlight the effect of stacking and its ability to prolong the survivability of the SARS-CoV-2 virus.

Based on the materials' porous cellulose composition, liquid disinfection methods may not be suitable and may result in material degradation. Longer quarantine time can be considered; or, other methods such as application of heat may promote more rapid decontamination and may warrant further investigation. A literature review that will be available in October 2020 is exploring published research on the effect of heat, UV light, and other methods of disinfection. Organizations such as the Northeast Document Conservation Center have shared information on disinfecting materials for archives, libraries and museums.

Test Methods

The items studied in Test 4 were not sterilized before testing. Battelle propagated the clinical isolate of the SARS-CoV-2 virus in-house, followed by characterization and testing to establish a certified titer. All testing was conducted within a biosafety level (BSL)-3 laboratory.

Test coupons (N=5) and blank (N=1), per timepoint, were excised from each of the five library or museum materials in 1.9 cm × 7.6 cm–sized coupons. Stock SARS-CoV-2 was applied as 10 10-µL droplets (100 µL total) on each coupon and allowed to dry at ambient laboratory conditions in a Class II biosafety cabinet (BSCII), as shown in Figure 1. This method and volume of inoculum is consistent with previous attenuation testing methods developed by Battelle[1] and allows for a controlled method of drying to allow for a consistent starting number of virus. Once dry, a set of test coupons were collected and processed (T0 samples), and the remainder of test coupons were moved to a Class III biosafety cabinet to maintain the desired ambient environmental conditions of 22 ± 2°C and relative humidity (RH) of 40 ± 10. Actual conditions achieved were 21.8 ± 0.30°C and 38.6 ± 1.84% RH. All material coupons, after inoculation and subsequent drying, were placed on top of a stainless steel rack or stacked onto like material types and placed into a sealed, environmentally controlled chamber for testing. This chamber does not have mixing fans and does not transmit light, so the test materials were not exposed to airflow or light while in the chamber. Due to the wavy nature of the softcover book and plastic protective cover, a section of book pages was used for each, after stacking, to promote flat surface contact between the materials.

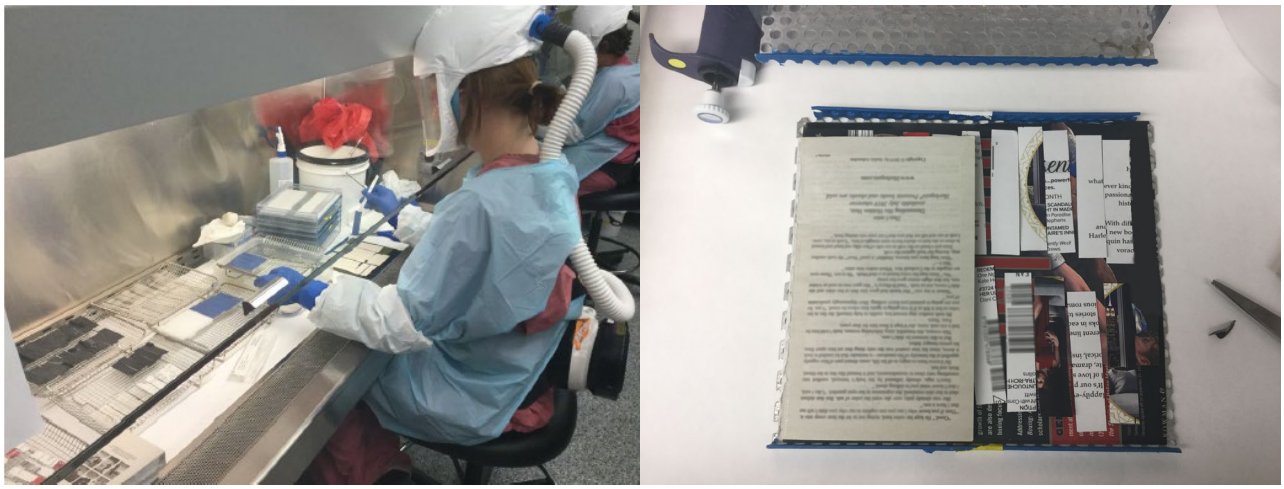
At the specified time points, the test coupons were removed from the environmental chamber and placed in 50-mL conical tubes (Fisher Scientific Cat. No. 14-959-49A, Waltham, MA, USA) and extracted with 10-mL complete cell culture media (Dulbecco's Modified Eagle Medium, Corning Cat. No. 10-010-CV, Corning, NY, USA), supplemented with 2% fetal bovine serum (Gibco Cat. No. 10082147, Carlsbad, CA, USA) and penicillin-streptomycin (Gibco Cat. No. 15140122), and agitated on a platform shaker at 200 rotations per minute for 15 minutes.

During the extraction process, there was a potential for chemicals from the test materials or adhesives contained within those materials to leach into the extracted liquid. Those chemicals could have had a

1 Richter, W.R, M.M. Sunderman, M.Q.S. Wendling, S. Serre, L. Mickelsen, R. Rupert, J. Wood, Y. Choi, Z. Willenberg, and M.W. Calfee. 2019. "Evaluation of altered environmental conditions as a decontamination approach for nonspore-forming biological agents." *Journal of Applied Microbiology* 128(4): 1050-1059. https://doi.org/10.1111/jam.14532.

deleterious cytopathic effects (CPE) on the cell culture monolayer. Since cell culture monolayers are needed for the median tissue culture infectious dose [$TCID_{50}$] assay to quantitatively determine infectious virus, it is important that the extractant does not have components other than the SARS-CoV-2 that will cause CPE, since this will result in false positives (i.e., presence of infectious virus).

Figure 1. Inoculation of SARS-CoV-2 onto Test 4 materials (left). After inoculation, the test coupons were placed inside the exposure chamber and stacked onto like material (right).



To mitigate the potential for chemically induced CPE, the extracts were transferred to a concentrator (Spin-X UF Concentrator, Corning Cat. No. CLS431491) and centrifuged until the ~10 mL starting volume was concentrated to ~ 0.5 mL. Approximately 10 mL of fresh complete cell culture media was added to the concentrated sample (i.e., extracts) for the purpose of washing and removing any residual chemicals. The concentrator was centrifuged again and concentrated to ~ 0.5 mL. Media was added to equilibrate all washed extracts to approximately 2 mL.

The limit of quantitation (LOQ) of this assay is 13.1 $TCID_{50}$ units. 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 a value of 10 (indicating positive) to allow it to be resolved from 0 (indicating negative) presence of viral infection in the Vero cells.

The test sample extracts were assayed in Vero E6 cells (ATCC CRL-1586, Manassas, VA, USA), and after a 72-hour incubation at 37°C with 5% CO_2, the $TCID_{50}$ assay plates were observed for CPE. The test matrix covered five time points (T, or day): T0, T2, T3, T4, and T6. As shown in Table 2 and Figure 2, at T0, a 1.3 to 1.9 log reduction (LR) was observed on all materials. Once dry and due to the stacked nature of the material, the rate of attenuation slowed and was still detectable through day 6. The expanded polyethylene foam, although not stacked, also resulted in recoverable virus through day 6.

Table 2. Test 4 total log_{10} SARS-CoV-2 recovered at days 0, 2, 3, 4 and 6.

Description	Inoculum[1]	T0[2]	2 Day	3 Day	4 Day	6 Day
Hardcover book cover	4.85	3.27	3.10	2.52	1.59	1.88
Softcover book cover	4.85	2.94	1.96	1.20	0.00	0.52
Plastic protective cover	4.85	3.53	2.29	1.83	1.30	1.30
DVD case	4.85	3.36	2.72	1.79	1.08	1.04
Expanded polyethylene foam	4.85	3.13	2.55	1.79	0.52	0.26

[1] Total number of virus applied to each material
[2] Total number of virus recovered after ~1hr dry period

Figure 2. Test 4 attenuation of SARS-CoV-2 at days 0, 2, 3, 4, and 6, with ± 95% confidence intervals indicated by the black vertical bars for each test date and item.