REALM REOpening Archives, Libraries and Museums PROJECT

Test 5: Natural attenuation as a decontamination approach for SARS-CoV-2 on textile materials

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. As part of this research, the 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. Information from REALM project test results should not be construed as recommendations or guidelines.

These findings contribute to the evolving scientific understanding regarding SARS-CoV-2, which still includes uncertainties about: how much virus is shed by an infected person through coughing, sneezing, talking, breathing, etc; how much virus is needed to infect someone; and the likelihood of a person becoming infected indirectly through contact with contaminated objects and surfaces ("fomites").

During Phases 1 and 2, Battelle has conducted five laboratory studies to study natural attenuation as a decontamination approach for materials contaminated with SARS-CoV-2. The results of Tests 1 through 4 were released on June 22, July 20, August 18, September 3, 2020, respectively; Test 5 began on August 29, 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 5 included five commonly used textile items; all items tested are listed in Table 1 below. The nylon webbing was provided by the American Museum of Natural History and the leather book was provided through private donation. The other materials were procured as samples from vendors. Samples from each item were inoculated and then allowed to dry. The items were then examined two, four, six, and eight days after an initial evaluation. Day eight was the final timepoint tested.

Table 1. Test 5 items examined.

Item	Material Type	Use
Leather book cover*	Leather (circa 1861)	Hardcover book covering
Synthetic leather*	Expanded polyvinyl chloride (PVC)	Upholstery
Polyolefin fabric*	100% polyolefin	Upholstery
Cotton fabric*	100% cotton (blue)	Upholstery, costumes
Nylon webbing**	Nylon weave	Nylon belt crowd control barrier

Results show that after eight days of quarantine, SARS-CoV-2 virus was still detected on leather and synthetic leather materials. For the leather book cover, the virus had dropped below the LOQ (1.42 $\log_{10}$, or 26.2 virus particles) by day 4, but the presence of virus was found on three of the five

test coupons on day 8. For the synthetic leather, the virus dropped below the LOQ by day 8 but was still found on two of the five test coupons. For the polyolefin fabric and nylon webbing, only the amount of virus after the initial 1 hour of drying time could be measured: fewer than 131 particles detected on the polyolefin and fewer than 655 on the nylon webbing. No data for the cotton fabric could be collected or reported.

Cells used for this TCID50 assay can die from two main effects: (1) *cytotoxicity*, which is cell death caused by an extracted chemical from the test coupon, or (2) *cytopathic effect* (CPE), which is cell death caused by the infectious virus extracted from the test coupon. Three of the tested materials—polyolefin fabric, nylon webbing, and cotton fabric—all showed some level of cytotoxicity, which limited the ability to measure the amount of virus at lower levels. The source of cytotoxicity from these three materials is unknown, but may be a coating (e.g., fire retardant or antimicrobial) or some other unknown manufacturing by-product.

This cytotoxicity led the researchers to adjust the limits of quantitation (LOQ) to a higher threshold for the polyolefin fabric and nylon webbing; below these levels no virus was able to be measured. The cotton material showed varying levels of cytotoxicity that affected multiple dilutions. This led to inconclusive data for cotton, and the researchers excluded the data from reporting.

Test Methods

The items studied in Test 5 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. A more detailed description of the test methods has been published on the REALM website[1].

Test coupons (N=5) and blank (N=1), per timepoint, were excised from each of the five 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 and allows for a controlled method of drying to allow for a consistent starting number of virus. [2] Once dry, a set of test coupons were collected and processed (T0 samples), and the remaining 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 during Test 5 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 and placed into a sealed environmentally controlled chamber for testing. This chamber did not have mixing fans and was not light transmissible; that is, test materials remained in the dark during exposure.

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

[1] Test Plan for the Natural Attenuation of SARS-CoV-2 as a Decontamination Approach, revised July 29, 2020, published to http://oclc.org/realm

[2] Richter W, Sunderman M, Wendling M, Serra S, Mickelsen L, Rupert R, Wood J, Choi Y, Willenberg Z, Calfee M (2019). Evaluation of altered environmental conditions as a decontamination approach for non-spore-forming biological agents. *Applied Microbiology* JAM-2019-0811

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) agitated on a platform shaker at 200 rotations per minute for 15 minutes.

The limit of quantitation (LOQ) of this assay is 26.2 $TCID_{50}$ units (1.42 log_{10}) when no cytotoxic effects are observed. 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. An average is calculated for the values assigned to the five test coupons for each material.

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.

Figure 1. Inoculation of SARS-CoV-2 onto Test 5 materials.

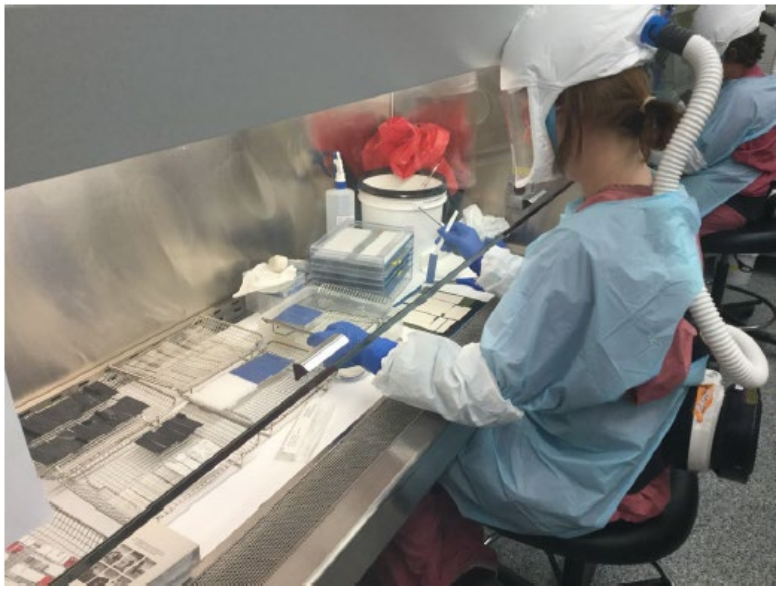
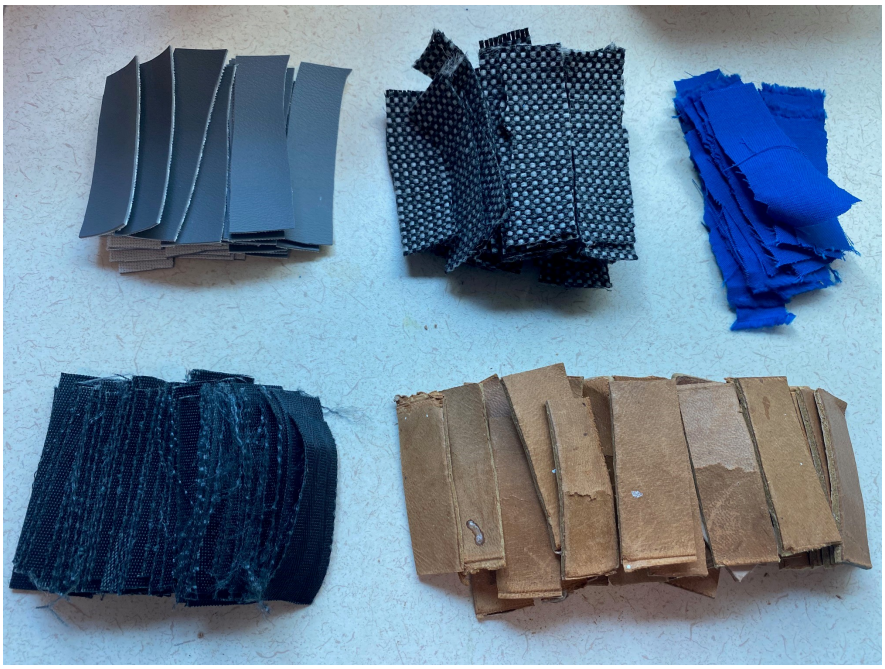


Figure 2. Test 5 test coupons for the synthetic leather (top left), polyolefin (top middle), cotton (top right), nylon (bottom left), and leather book cover (bottom right).



During the extraction process, there exists a potential for chemicals from the test materials or adhesives contained within those materials, to leach into the extracted liquid. 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 cytopathic effects, since this will result in false positives (i.e., cell death due to leached chemicals in test material and not due to virus infection). The negative control test samples (i.e., test coupons not inoculated with SARS-CoV-2) control for cytotoxic effect and are used for each material type. Three of the test materials (polyolefin, nylon, and cotton) for Test 5 resulted in extracted chemicals that resulted in cytotoxic effects on the cell culture monolayer. Polyolefin resulted in cytotoxic effect in the undiluted samples only, while nylon resulted in cytotoxic effect at both the undiluted row as well as the first 5-fold dilution on the test plate. Cotton resulted in cytotoxicity at multiple dilutions throughout the assay and as a result was excluded from analysis. As dilutions (per material) are excluded from the test, due to cytotoxic effect, the LOQ must be adjusted, as the ability to measure infectious virus at those dilutions is negated. The LOQ was adjusted from 1.42 log_{10} to 2.12 log_{10} for the polyolefin and to 2.82 log_{10} for the nylon.

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, T4, T6 and T8. As shown in Table 2 and Figure 3, at T0, a 1.0 to 3.3 log reduction (LR) was observed on all materials. Once dry, the rate of attenuation slowed and was still detectable through day 8. The leather book cover as well as the synthetic leather resulted in recoverable infectious virus through day 8. The polyolefin and nylon material resulted in no

detectable virus after 1 hour of drying, within the adjusted LOQ (ALOG) of 2.12 log_{10} and 2.82 log_{10}, respectively. Below these adjusted LOQ, no qualitative analysis was achievable due to the complication of cytotoxic effects.

Table 2. Test 5 total log_{10} SARS-CoV-2 recovered at days 0, 2, 4, 6 and 8.

Description	Inoculum[1]	T0[2]	2 Day	4 Day	6 Day	8 Day
Leather book cover	5.40	2.99	2.02	0.91	0.78	0.78
Synthetic leather	5.40	4.42	2.88	2.45	1.76	0.52
Polyolefin fabric	5.40	<2.12	<2.12	<2.12	<2.12	<2.12
Cotton fabric	5.40	NA[3]	NA[3]	NA[3]	NA[3]	NA[3]
Nylon webbing	5.40	<2.82	<2.82	<2.82	<2.82	<2.82

[1] Total number of virus applied to each material
[2] Total number of virus recovered after ~1hr dry period
[3] Material excluded due to cytotoxic effect at multiple dilutions

Figure 3. Test 5 attenuation of SARS-CoV-2 at days 0, 2, 4, 6, and 8, with ± 95% confidence intervals indicated by the black vertical bars for each test date and item.