REALM PROJECT
REopening Archives, Libraries, and Museums

Test 3: Natural attenuation as a decontamination approach for SARS-CoV-2 on five plastic-based 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 archive, 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 research, Battelle has conducted three natural attenuation studies to provide information on how long some commonly handled library materials would need to be considered for quarantine prior to being put back into use. The results of Test 1 and Test 2 were released on June 22 and July 20, 2020, respectively; Test 3 began on July 10, 2020. The studies were 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 3 included the five items listed in Table 1.

Table 1. Test 3 items examined.

Item	Material type	Use
Talking book, USB cassette*	Acrylonitrile butadiene styrene (ABS), specific blend	Cartridges are used in talking book readers available through the National Library Services for the Blind and Disabled
DVD**	Polycarbonate	Digital data storage (also includes CDs). Note: A polypropylene DVD case was tested in Test 1.
Storage bag (flexible plastic)**	Low-density polyethylene (LDPE), recycling #4	Storage, library and museum kits, gift shop packaging
Storage container (rigid plastic)**	High-density polyethylene (HDPE), recycling #2	Transporting and storage of items
Plexiglass ***	Acrylic	Display cases, partitions

Items were provided by the National Library Service for the Blind and Print Disabled, Library of Congress*; Columbus Metropolitan Library**; and the National Archives and Records Administration***. Samples from each item were inoculated and placed on top of a stainless steel rack. In contrast to Test 2, these items were *not* tested in a nested (or stacked) configuration to mimic common operating procedures.

Results show that after five days of quarantine in an unstacked configuration, the SARS-CoV-2 virus was not detected on the storage bag (flexible plastic) or the DVD. The storage container (rigid plastic), plexiglass, and the USB cassette all showed detectable virus at five days. Day five was the final timepoint tested.

Compared to the results of Test 1 and 2, this data suggests that a slightly longer quarantine time for these types of plastic-based materials may be required to render SARS-CoV-2 undetectable through natural attenuation alone. **Alternatively, based on the materials' nonporous nature, suitable liquid disinfection methods may promote a more rapid decontamination than the quarantine method**.

Test Methods

The items studied in Test 3 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 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. 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 21.9 ± 0.61°C and 37.4 ± 0.92% RH. All material coupons, after inoculation and subsequent drying, were placed on top of a stainless steel rack and into the environmentally controlled chamber for testing.

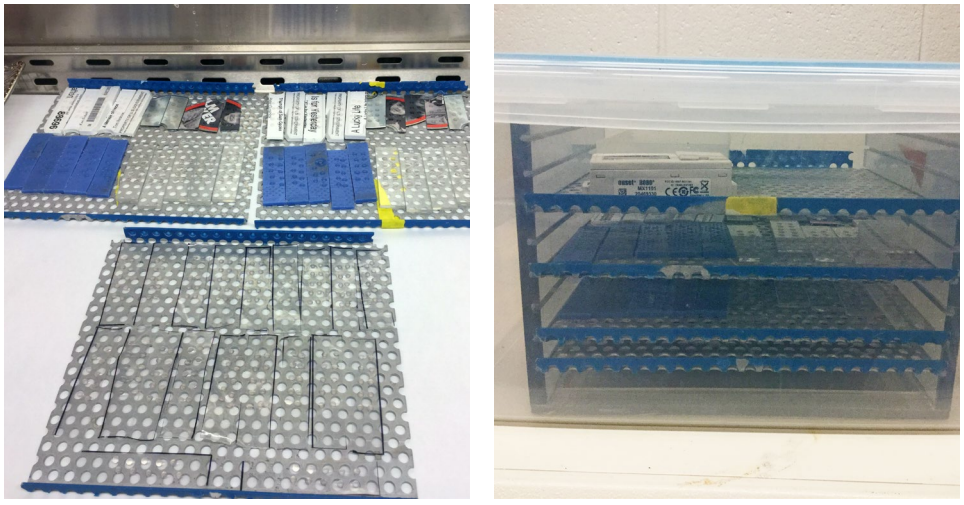

Figure 1. Inoculation of SARS-CoV-2 onto Test 3 materials (left). After inoculation, the extracted test coupons were placed inside the exposure chamber to control Temp and RH (right).

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) 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 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).

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 T5. As shown in Table 2 and Figure 3, at T0, a 1.2 to 1.5 log reduction (LR) was observed on all materials. Once dry, the rate of attenuation slowed and by day 5, two materials (the storage bag and DVD) had attenuated below the level of detection for the assay, meaning no CPE was observable in the undiluted extract placed onto the Vero cells. Recoverable SARS-CoV-2 were still observable on the USB cassette, the storage container, and the plexiglass through day 5, although all were below the LOQ.

Table 2. Test 3 total log_{10} SARS-CoV-2 recovered at days 0, 2, 3, 4, and 5.

Description	Inoculum[1]	0 Day[2]	2 Day	3 Day	4 Day	5 Day
Talking book, USB cassette	4.70	3.24	1.45	1.12	0.54	0.26
DVD	4.70	3.24	1.28	1.55	1.39	< LOD
Storage bag (flexible plastic)	4.70	3.47	1.76	1.77	0.52	< LOD
Storage container (rigid plastic)	4.70	3.16	1.26	0.85	0.78	1.04
Plexiglass	4.70	3.24	1.41	1.61	0.52	0.52

[1] Total number (log_{10}) of virus applied to each material
[2] Total number (log_{10}) of virus recovered after ~1hr dry period

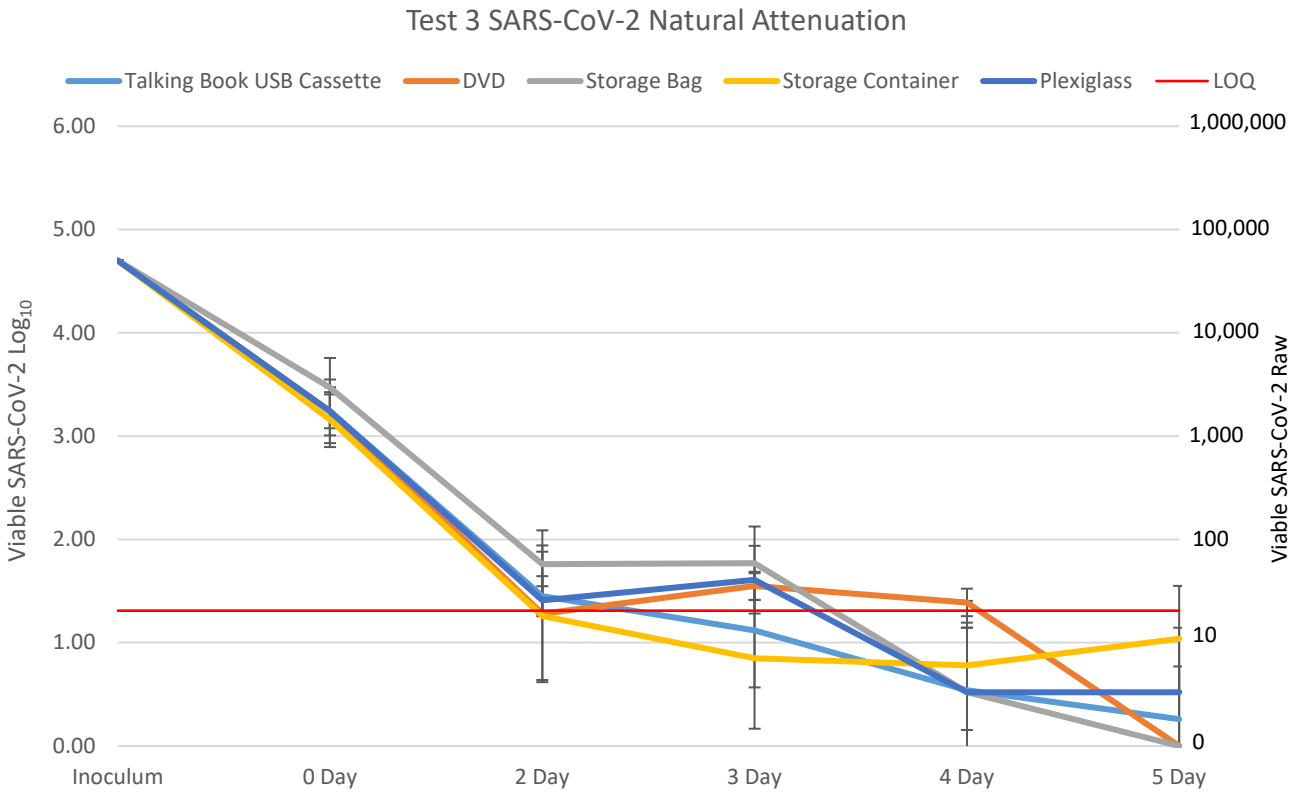

Figure 3. Test 3 attenuation of SARS-CoV-2 at days 0, 2, 3, 4, and 5, with ± 95% confidence intervals indicated by the black vertical bars for each test date and item.