

Test 2: Natural attenuation as a decontamination approach for SARS-CoV-2 on five paper-based library and archives 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 create and distribute science-based information designed to reduce the risk of transmission of COVID-19 to staff and visitors who are engaging in the delivery or use of museum, library, and archival 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 two natural attenuation studies to provide information on how long some commonly handled library materials would generally need to be considered for quarantine in order for the virus to be undetectable. The [results of Test 1](#) were released on June 22, 2020; Test 2 began on June 23, 2020. The studies were conducted by applying the virulent SARS-CoV-2 virus on five materials held at standard room temperature and humidity conditions. The materials in Test 2 included the following five items, which 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***:

1. Braille paper pages*
2. Glossy book pages**
3. Magazine pages**
4. Children's board book**
5. Archival folders***

Samples from each item were inoculated and placed inside the closed book or magazine. The items were then configured to mimic common storage conditions such as stacked or shelved books, or a pile of folders or magazines. (In Test 1, the items were not stacked.)

Results show that after two days of quarantine in a stacked configuration, the SARS-CoV-2 virus was not detectable on the archival folders.

After four days of quarantine in their stacked configuration, the virus was not detectable on the braille pages, glossy book pages, and board book.

The magazine pages showed a trace amount of virus at four days. Day four was the final timepoint tested.

This evaluation indicated that standard office temperature (68°F to 75°F) and relative humidity conditions (30 to 50 percent) may provide an environment that allows for the natural attenuation of

SARS-CoV-2 present on these materials after two days of quarantine for archival folders and **four days of quarantine for the book pages**. Compared to the results of Test 1, the results of Test 2 indicate that a longer quarantine time for these types of cellulose-based paper materials may be required to render SARS-CoV-2 undetectable.

Test Methods

The items studied in Test 2 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 21.8 ± 0.48°C and 42.8 ± 1.89% RH. All material coupons, after inoculation and subsequent drying, were placed back into the item from which they were collected, and the entire book or stack of material was placed into the environmentally controlled chamber for testing.



Figure 1. Inoculation of SARS-CoV-2 onto test materials (magazine–left; children’s board book–right). After the inoculation, the extracted test coupons were placed back inside the closed item.

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 extraction process there was a potential for chemicals from the test materials, or adhesives contained within those materials, to leach into the extract 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 [TCID₅₀] 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 cytopathic effect, the extracts were transferred to a concentrator (Spin-X UF Concentrator, Corning Cat. No. CLS431491) and centrifuged until the approximately 10 mL starting volume was concentrated to approximately 0.5 mL. Approximately 10 mL fresh complete cell culture media was added to the concentrated sample (i.e., extracts) for the purpose of washing and removing any residual chemicals. Media was added to equilibrate all washed extracts to approximately 2 mL.

The limit of quantitation (LOQ) of this assay is 13.1 TCID₅₀ 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 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% CO₂, the TCID₅₀ assay plates were observed for CPE. The test matrix covered five time (T, or day) points: T0, T1, T2, T3, and T4. As shown in Figures 2 and 3, at T0 a 2 to 4 log reduction (LR) was observed on all materials. Once dry, the rate of attenuation slowed and by day 4, all but magazine pages had attenuated below the level of detection for the assay, meaning no CPE was observed in the undiluted extract placed onto the Vero cells. While undetectable at day 3, trace amounts of SARS-CoV-2 were still observable on magazine test material at day 4. The reemergence of detectability on magazine pages at day 4 was the result of positive detection of virus (below LOQ) on only one of the five test coupons, which indicates low levels of persistence.

Total Log ₁₀ SARS-CoV-2 Recovered						
Description	Inoculum ¹	T0 ²	1 Day	2 Day	3 Day	4 Day
Children's Board Book	5.26	2.55	1.30	1.06	0.78	< LOD
Archival Folder	5.26	1.30	0.87	< LOD	< LOD	< LOD
Braille Paper Page	5.26	1.82	0.82	0.78	0.26	< LOD
Glossy Book Page	5.26	3.16	2.45	0.87	0.57	< LOD
Magazine Page	5.26	2.13	1.31	0.26	< LOD	0.26
¹ Total number of virus applied to each material						
² Total number of virus recovered after ~1hr dry period						

Figure 2: Total Log₁₀ SARS-CoV-2 Recovered at days 1, 2, 3 and 4

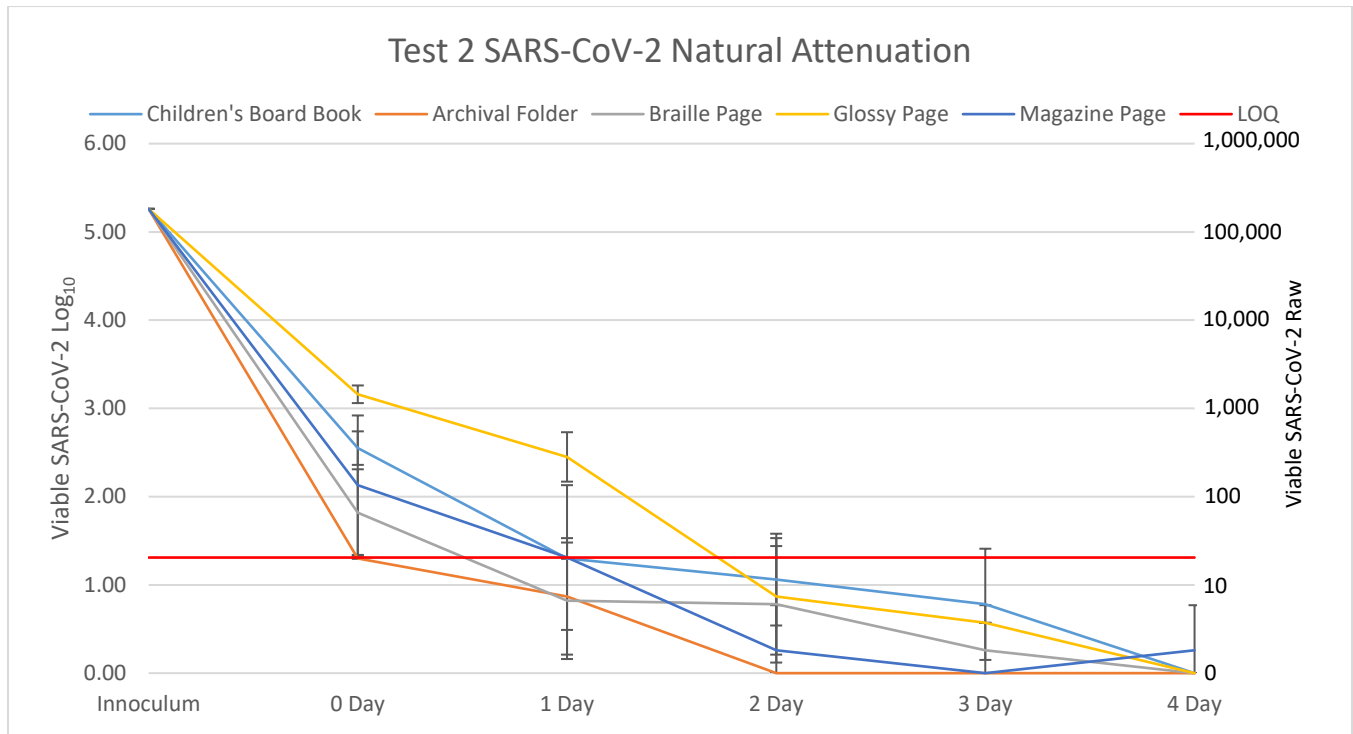


Figure 3. Test 2 attenuation of SARS-CoV-2 at days 1, 2, 3, and 4 ± 95% confidence interval. The [confidence intervals](#) are indicated by the black vertical bars for each test date and item.