REALM PROJECT REopening Archives, Libraries and Museums

Test 7 and 8: Natural attenuation as a decontamination approach for SARS-CoV-2 on materials at various temperatures

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").

Battelle has conducted eight laboratory studies to evaluate natural attenuation (*i.e.*, inactivation) as a decontamination approach for materials contaminated with SARS-CoV-2. The results of Tests 1 through 6 were released between June 22 and November 19, 2020; Tests 7 and 8 began concurrently on January 5, 2021.

Tests 1 through 6 measured the attenuation time of virulent SARS-CoV-2 virus applied to materials held at ambient room temperature (68 to 75°F; 20 to 24°C) and relative humidity (RH) conditions (30 to 50 percent). For Test 7, materials were held at colder (34 to 36°F; 1 to 4°C) temperatures; for Test 8, materials were held at warmer (83 to 84°F; 28 to 29°C) temperatures. For both tests, relative humidity remained the same as previous tests. The tests studied three types of book covers and expanded polyethylene foam; see Table 1. Items were provided by the Columbus Metropolitan Library* and the National Archives and Records Administration**. The book cover materials were tested in a stacked configuration, the foam in an unstacked configuration. The Test 7 materials (colder temperature) were examined on days 2, 6, 8, 9, and 10; Test 8 materials (warmer temperature) were examined on days at 2, 3, 4, 6, and 8.

Table 1. Test 7 and 8 materials

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
Expanded polyethylene foam**	1-in. polyethylene foam	Storage and shipping

Results show that attenuation rates for materials held at the colder temperature were significantly slower compared to the warmer and ambient temperatures. At day 10, the final Test 7 timepoint, the amount of active virus present remained nearly unchanged from the T0 measurement for all materials

except hardcover book cover. In contrast, in Test 8, by day 6 the virus was undetected on all materials except the plastic protective cover; this was a slightly faster attenuation time than what occurred at ambient temperatures. The virus was undetectable on the plastic protective cover at day 8. This data may suggest that additional considerations may need to be evaluated regarding outdoor collection boxes, or storage in colder conditions. For institutions using quarantine periods, this research can impact when to start the quarantine "clock" once a material is brought into a controlled environment. Data also suggests that, when possible, storage in warmer areas may help to shorten the length of quarantine.

Note: Leather had also been selected as a test material for Tests 7 and 8. However, during pre-screening of the samples (a leather book cover and two types of bulk leather), cytotoxicity – that is, cell death caused by a chemical extracted from the material (described below) – was observed. Because it would prevent researchers from measuring the amount of virus in the material during the test, leather was removed from the tests. Leather was previously tested as part of the REALM study (Test 5) without observed cytotoxicity. That leather was collected from a book from the 1860s, while the leathers selected for Tests 7 and 8 were newer. It is suspected that the modern tanning process or dyes used in manufacturing of these newer leathers may have resulted in the cytotoxic effect, however, the exact cause remains undetermined.

Test Methods

The items studied in Tests 7 and 8 were not sterilized before testing. Battelle propagated the clinical isolate of the SARS-CoV-2 virus (strain WA1/2020) 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) sized at 1.9 cm × 7.6 cm, per timepoint, were excised from each of the four materials. Stock SARS-CoV-2 was applied as ten 10-µL droplets (100 µL total) on each coupon and allowed to dry at ambient laboratory conditions in a Class II biosafety cabinet, 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 for a consistent starting number of

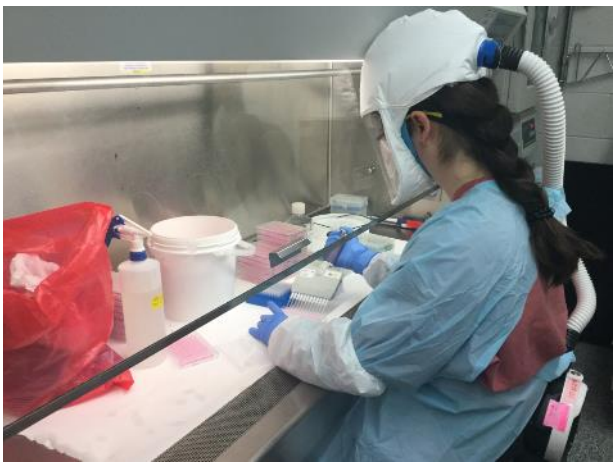

Figure 1. Inoculation of SARS-CoV-2 onto Test 7 and 8 materials.

[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

infectious virus.[2] Once dry, a set of test coupons were collected and processed (T0 samples; Tables 2 and 3), the remaining coupons were then placed into contact with like surfaces (stacked configuration) to mimic typical storage conditions for three of the four materials (hardcover book cover, softcover book cover, plastic protective film); expanded polyethylene foam was not tested in a stacked configuration. The test coupons (stacked or unstacked) were then arranged on top of stainless steel racks and placed into their respective environmentally controlled chamber for testing at the desired environmental conditions. Average temperatures for Test 7 were 36.3 ± 2.40°F (2.4 ± 1.36°C), with 34.5 ± 3.58% RH; temperatures for Test 8 were 83.5 ± 0.62°F (28.6 ± 0.32°C), with 32.6 ± 0.48% RH. These chambers did not have mixing fans and were not light transmissible; that is, test coupons remained in the dark during exposure.

At the specified timepoints, the test coupons were removed from the environmental chambers 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.

The limit of quantitation (LOQ) of this assay is 26.2 median tissue culture infectious dose ($TCID_{50}$) 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 E6 host cells. An average is calculated for the values assigned to the five test coupons for each material per timepoint.

Figure 2. Material extraction processing

During the extraction process, shown in Figure 2, there exists a potential for chemicals from the test materials or adhesives contained within those materials, to leach into the extracted liquid.

[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

Cells used for this $TCID_{50}$ 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.

Since cell culture monolayers are needed for the $TCID_{50}$ 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).

To mitigate potential cytotoxic effects, 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 (*i.e.*, a buffer exchange). The concentrator was centrifuged again and concentrated to ~ 0.5 mL. Media was added to equilibrate all washed extracts to approximately 2 mL.

Negative control test samples (*i.e.*, test coupons not inoculated with SARS-CoV-2) control for CPE and are used for each material type. None of the materials use for Tests 7 and 8 resulted in leached chemicals that resulted in CPE on the cell culture monolayer, with noted exception of leather, which was prescreened and excluded from testing.

The test sample extracts were assayed in Vero E6 cells (ATCC CRL-1586, Manassas, VA, USA), and after a 72-hour incubation at 99°F (37°C) with 5% CO_2, the $TCID_{50}$ assay plates were observed for CPE. The test matrix covered six time points per test condition (T, or day): T0, T2, T6, T8, T9 and T10 for cold test condition (Test 7), and T0, T2, T3, T4, T6 and T8 for warm test condition (Test 8). As shown in Tables 2 and 3, at T0 (after 1 hour of drying), a 1.1 to 1.5 log reduction (LR) was observed on all materials which was consistent with previous LR values for these material types (Test 4). Since Test 7 and 8 were conducted concurrently, T0 data was performed with one set of test materials and data shared across both tests.

Table 2. Test 7 (cold temperature) total log_{10} SARS-CoV-2 recovered at days 0, 2, 6, 8, 9 and 10.

Description	Inoculum[1]	T0[2]	2 Day	6 Day	8 Day	9 Day	10 Day
Hardcover book cover	4.71	3.48	3.65	3.22	3.75	< LOD	1.16
Softcover book cover	4.71	3.24	3.46	3.38	3.86	3.47	3.41
Plastic protective cover	4.71	3.64	3.80	3.66	3.22	3.44	3.13
Foam	4.71	3.52	3.78	3.22	2.88	3.22	2.88

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

Table 3. Test 8 (warm temperature) total log_{10} SARS-CoV-2 recovered at days 0, 2, 3, 4, 6 and 8.

Description	Inoculum[1]	T0[2]	2 Day	3 Day	4 Day	6 Day	8 Day
Hardcover book cover	4.71	3.48	1.68	0.78	0.26	< LOD	< LOD
Softcover book cover	4.71	3.24	1.98	0.52	0.26	< LOD	< LOD
Plastic protective cover	4.71	3.64	2.09	1.30	0.52	0.78	< LOD
Foam	4.71	3.52	1.75	0.26	0.52	< LOD	< LOD

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

A summary of the attenuation results per material type are shown in Figures 3 through 6. Selected data from Test 4 was included (dashed line) in these data sets as a reference to the attenuation curves previously established for testing conducted at target ambient conditions [71.6°F (22°C) and 40% RH]. As expected, the attenuation rate for the cold condition materials was significantly slower compared to the warm condition as well as the ambient condition materials. Hardcover book cover was the only test material, at the cold test condition, that showed any significant attenuation, resulting in a reduction in viable virus by day 9 (<LOD) and remaining below LOQ at day 10, with two of five test coupons remaining positive for viable virus.

In contrast, the warm condition testing resulted in a less significant increase in the rate of attenuation as compared to the ambient condition testing. It is worth pointing out that the difference in temperature for cold and warm condition as compared to ambient testing was 35°F (20°C) and 12°F (7°C), respectively. Again, the hardcover book cover resulted in the most significant difference in rate of attenuation when compared to ambient and, although subtle, all materials except plastic protective cover resulted in 0 of 5 test coupons positive for viable virus at day 6 at the elevated test temperature. The plastic protective cover also resulted in 0 of 5 test coupons positive for viable virus, but at day 8. This is a notable endpoint improvement (compared to ambient) for all test materials, and although subtle, does suggest that even this modest 12°F (7°C) increase in temperature does improve the attenuation rate of SARS-CoV-2 on these materials.

While it is anticipated that more extreme elevated temperatures would result in faster attenuation rates, for the purposes of generating useful data that could be easily implemented in the community without the need for additionally purchased equipment, these elevated temperatures were selected because they are believed to be readily attainable through use of existing heating and ventilation equipment without the need for modification.[3]

[3] Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J. 2020 Oct 7;17(1):145. doi: 10.1186/s12985-020-01418-7. PMID: 33028356; PMCID: PMC7538848.

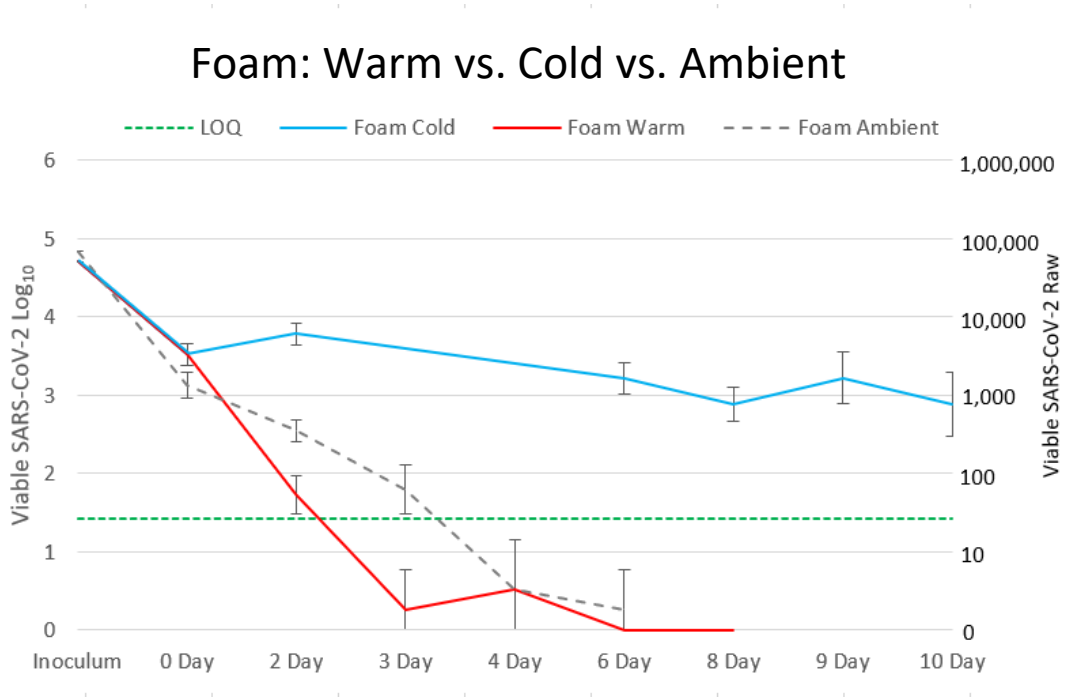

Figure 3. Attenuation of SARS-CoV-2 on foam at warm, cold, and ambient conditions ± 95% confidence intervals indicated by the black vertical bars for each test date and item.

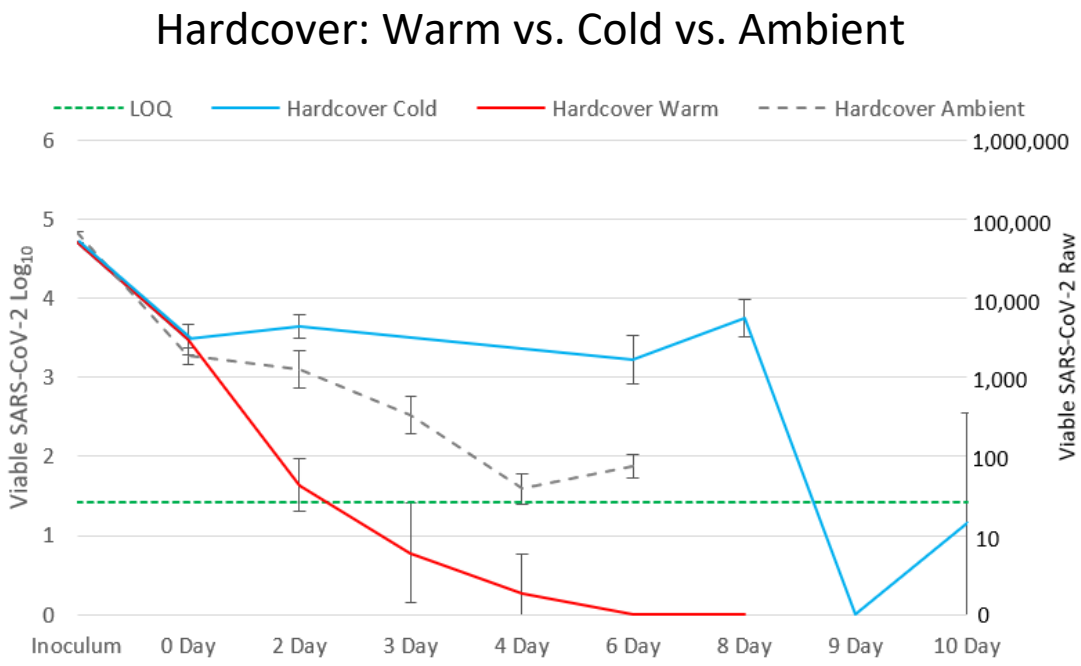

Figure 4. Attenuation of SARS-CoV-2 on hardcover book cover at warm, cold, and ambient conditions ± 95% confidence intervals indicated by the black vertical bars for each test date and item.

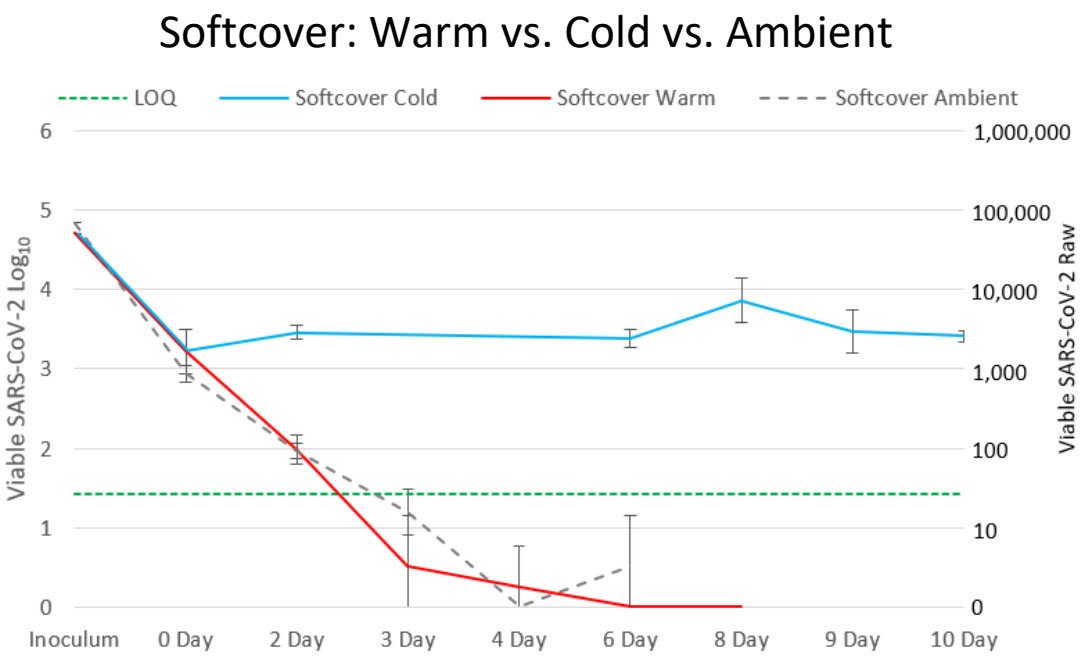

Figure 5. Attenuation of SARS-CoV-2 on softcover book cover at warm, cold, and ambient conditions ± 95% confidence intervals indicated by the black vertical bars for each test date and item.

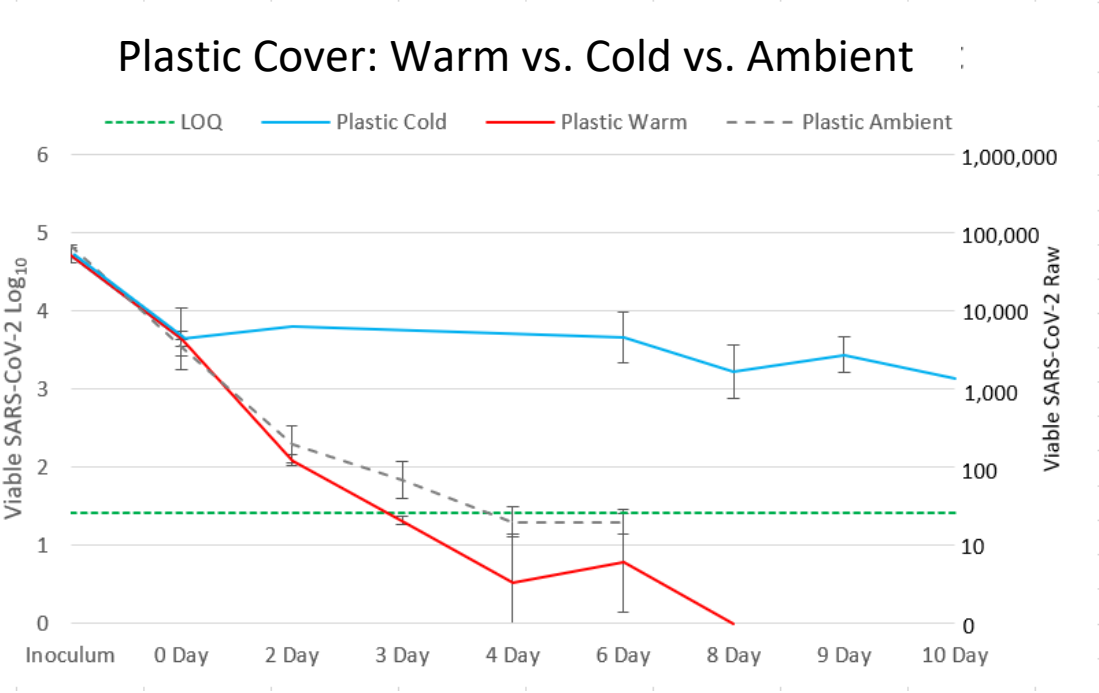

Figure 6. Attenuation of SARS-CoV-2 on plastic protective cover at warm, cold, and ambient conditions ± 95% confidence intervals indicated by the black vertical bars for each test date and item.