REALM PROJECT REopening Archives, Libraries and Museums

Test 1: Natural attenuation as a decontamination approach for SARS-CoV-2 on five library materials

Originally released, June 22, 2020 and updated, October 14, 2020.

Erratum:

The limit of quantitation (LOQ) for the $TCID_{50}$ assay being used for this testing has been corrected due to an error in the total volume of test material liquid extract being evaluated. The corrected LOQ is 26.2 $TCID_{50}$; it was previously reported at 13.1 $TCID_{50}$. Note that this change does not affect any of the reported SARS-CoV-2 datapoints generated.

Update:

Paragraph 2: A description of current uncertainties about SARS-CoV-2 in the published research has been added.

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.

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").

As part of the project's Phase 1 research, Battelle has conducted a natural attenuation study to provide information on how long some commonly circulated library materials would need to be considered for quarantined prior to being put back into public circulation. Testing was conducted by applying the virulent SARS-CoV-2 virus on five materials held at standard room temperature and humidity conditions. The materials tested include the following items, which were provided by Columbus Metropolitan Library:

1. Hardback book cover (buckram cloth),
2. Paperback book cover,
3. Plain paper pages inside a closed book,
4. Plastic book covering (biaxially oriented polyester film), and
5. DVD case.

Results show that the SARS-CoV-2 virus was not detectable on the materials after three days of quarantine. The evaluation demonstrates that standard office temperature and relative humidity conditions typically achievable by any air-conditioned office space provide an environment that allows for the natural attenuation of SARS-CoV-2 present on these common materials after three days of

quarantine. This report describes the results of the first test set, Test 1, which consists of test 1.1 and test 1.2.

Test Methods

The library materials provided by Columbus Metropolitan Library were not pre-sterilized before testing. Battelle grew the clinical isolate (USA-WA1/2020) of the SARS-CoV-2 virus in-house, followed by characterization and testing to establish concentration of the virus. 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 selected 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 ranged from an average of 21.9 to 22.9°C and 41.3 to 50.0% RH for tests 1.1 and 1.2, respectively. The plain paper coupons, after drying, were placed back into the book from which they were collected, and the entire book was placed into the environmentally controlled chamber for testing.

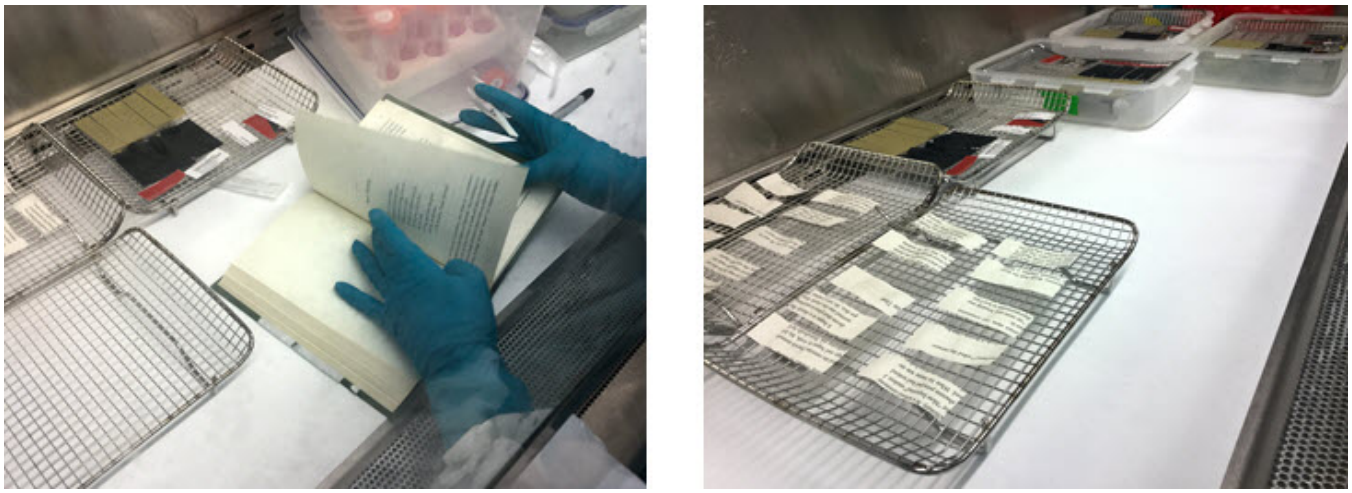

Figure 1. Inoculation of SARS-CoV-2 onto test materials.

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, Mass., US) and extracted with 10-mL complete cell culture media (Dulbecco's Modified Eagle Medium, Corning Cat. No. 10-010-CV, Corning, N.Y., US) supplemented with 2% fetal bovine serum (Gibco Cat. No. 10082147, Carlsbad, Calif., US) 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 [$TCID_{50}$] 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 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.*, retentate) for the purpose of washing and removing any residual chemicals. Media was added to equilibrate all washed retentates to approximately 2 mL.

The test sample retentates 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 initial test matrix (Test 1.1) was intended to cover three times (T, or day) points: T6, T9, and T12. As you can see in Figure 2, at 0 Day (T0) a 1 to 1.5 log reduction (LR) had occurred on most materials. Plain paper showed a more aggressive attenuation rate and dropped below the limit of quantitation (LOQ) of 26.2 TCID50 units at T0. By day 6, all samples had attenuated below the level of detection for the assay, meaning no CPE was observable in the undiluted extract placed onto the Vero cells.

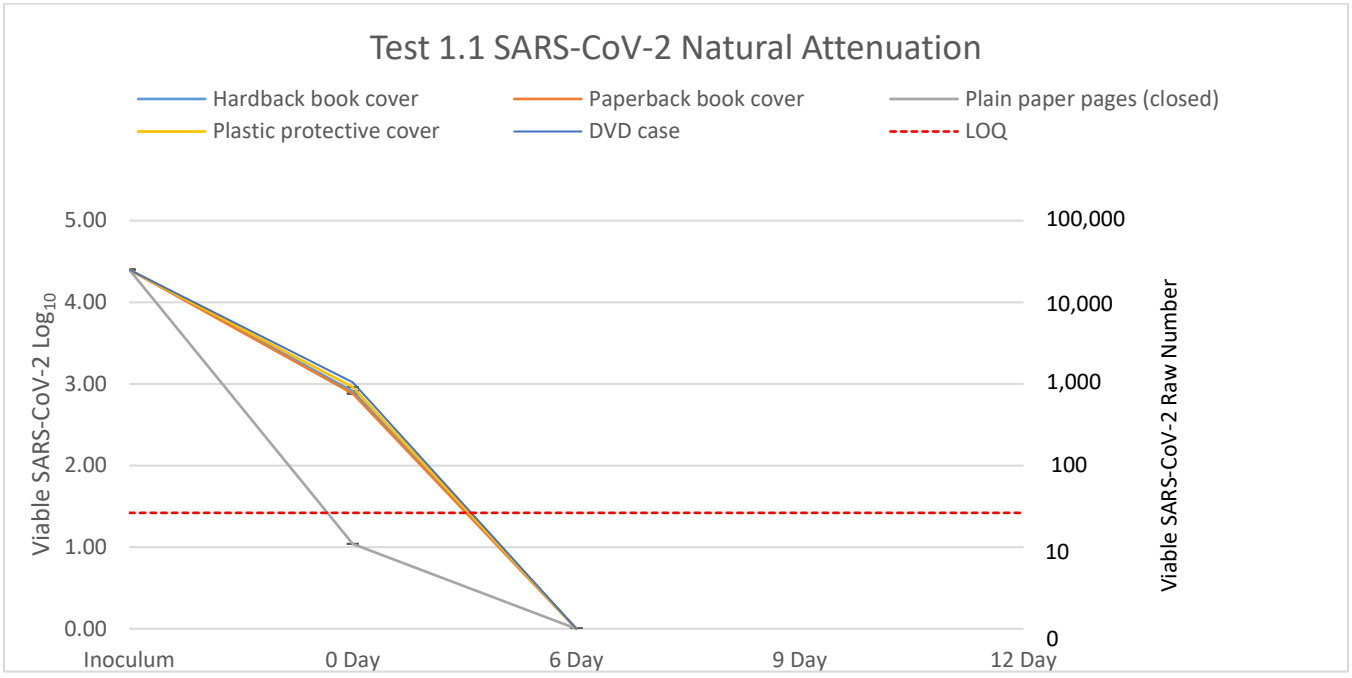

Figure 2. Natural attenuation of SARS-CoV-2 at days 6, 9, and 12 during test 1.1.

Since day 6 resulted in no detectable virus, test 1.2 was initiated to evaluate the materials at T0, T1, T3, and T4 to get better resolution of where complete attenuation was occurring. The virus stock used for test 1.2 had a higher initial titer, which resulted in a 1 log increase in organism applied to each test

material. As shown in Figure 3, a similar 1 to 1.5 log reduction was observed because of the drying/extraction process, however, the increased titer resulted in recoverable virus as compared to test 1.1, specifically on plain paper pages. **After one day of attenuation, there was no recoverable virus (below LOD) for the hardback book cover, the paperback book cover, or the DVD case. By day three, all five tested material surfaces resulted in no recoverable virus.**

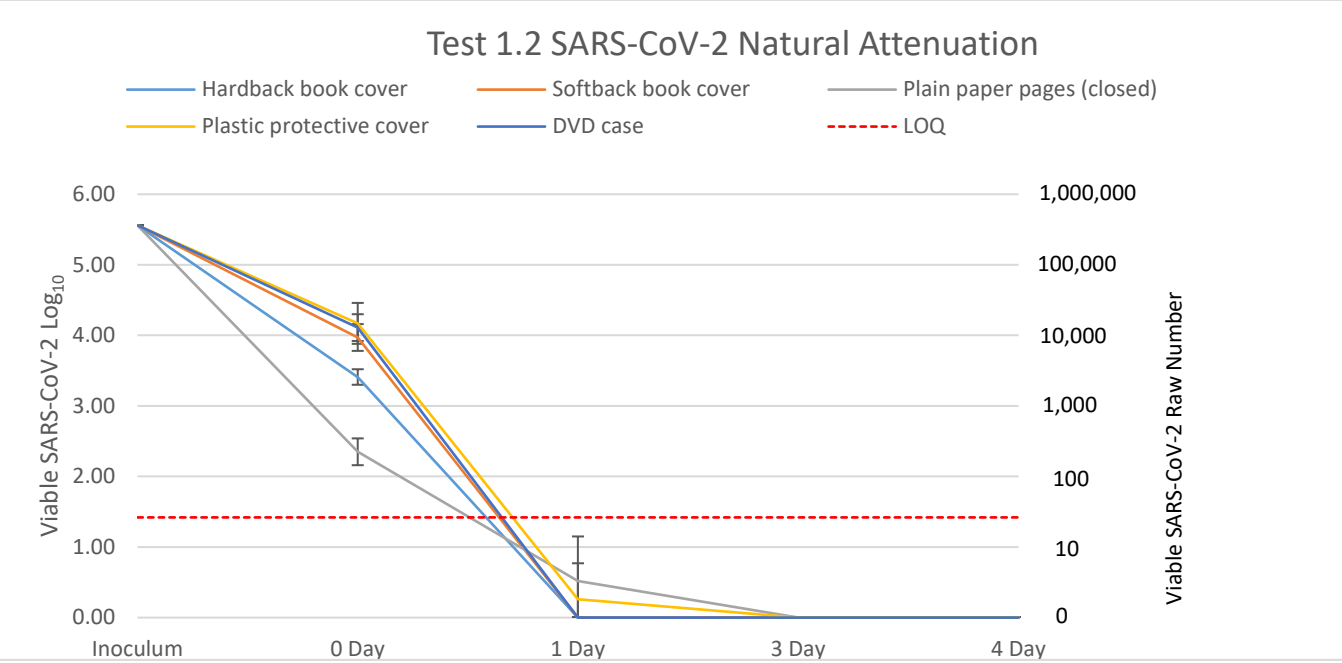

Figure 3. Natural attenuation of SARS-CoV-2 at days 1, 3, and 4 during test 1.2.