REALM REopening Archives, Libraries and Museums PROJECT

Test 6: Natural attenuation as a decontamination approach for SARS-CoV-2 on building 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 six 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 5 were released on June 22; July 20; August 18; September 3; and October 14, 2020, respectively. Test 6 began on October 8, 2020.

Each study has been conducted by applying the virulent SARS-CoV-2 virus on five materials held at ambient room temperature (68°F to 75°F) and relative humidity conditions (30 to 50 percent). The materials in Test 6 included five commonly used building materials; all items tested are listed in Table 1. The marble was provided by the National Park Service, the laminate was provided by Metropolitan New York Library Council, and the powder-coated steel was provided by the Library of Congress. The other materials were procured as samples from vendors. Test coupons were cut from each item, inoculated with active virus, and then allowed to dry. The test coupons were then examined two, four, six, and eight days (*i.e.*, timepoints) after the initial evaluation. Day 8 was the final timepoint tested.

Table 1. Test 6 materials

Item	Material Type	Use
Glass	Glass	Windows, doors, display cases
Marble	Danby marble	Flooring, counters, columns
Laminate	Laminate with particle board backing	Countertops
Brass	260 brass	Fixtures, railings
Powder-coated steel	Powder-coated steel	Lockers, shelving, book trucks, exhibit elements

Results show that after two days, SARS-CoV-2 virus was not detectable on the brass and marble. After six days, virus was not detected on the glass, laminate, and powder-coated steel. The brass resulted in the fastest attenuation rate: after one hour of drying, the virus was below the limit of quantitation (LOQ) and detectable on only three of the five brass test coupons. Based on the materials' nonporous nature, suitable liquid disinfection methods may promote a more rapid decontamination than the quarantine method. The U.S. Environmental Protection Agency (EPA) provides a **list of disinfectants and surface cleaners** that meet their criteria for use against SARS-CoV-2.

Test Methods

The items studied in Test 6 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) sized at 1.9 cm × 7.6 cm, per timepoint, were excised from each of the five 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 infectious virus.[2] Once dry, a set of test coupons were collected and processed (T0 samples; Table 2), and the remaining test coupons were moved to a Class III biosafety cabinet to maintain the desired ambient environmental conditions of 22 ± 2°C (71.6° F) and relative humidity (RH) of 40 ± 10. Actual average conditions achieved during Test 6 were 21.7 ± 0.14°C (71.06° F) and 36.6 ± 0.79% RH. All test 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 coupons remained in the dark during exposure.

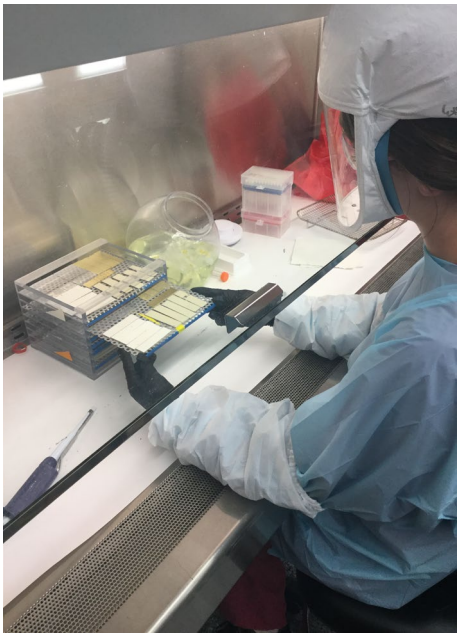

Figure 1. Inoculation of SARS-CoV-2 onto Test 6 materials.

At the specified timepoints, 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.

[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

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.

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. 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.

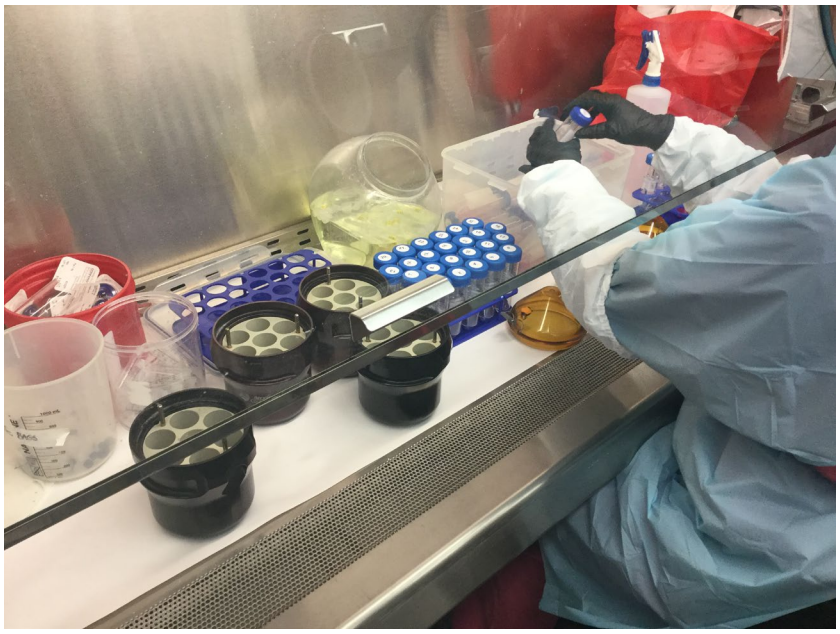

Figure 2. Test 6 material processing

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 used for Test 6 resulted in leached chemicals that resulted in CPE on the cell culture monolayer.

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 2.2 to 4.5 log reduction was observed on all materials. After one hour of drying (T0 timepoint), the virus was only detectable (below LOQ) on three of the five brass test coupons; after two days, the virus was not detectable on any of the brass or marble coupons. After four days, the virus was detectable (below the LOQ) on glass, laminate, and powder-coated steel resulting in 1 of 5, 1 of 5, and 3 of 5 positive test coupons, respectively. After six days of exposure no detectable virus remained on any of the five test materials.

Table 2. Test 6 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
Glass	5.26	2.96	1.41	0.26	< LOD	< LOD
Marble	5.26	3.08	< LOD	< LOD	< LOD	< LOD
Laminate	5.26	2.96	1.34	0.28	< LOD	< LOD
Powder-coated steel	5.26	2.94	0.56	0.78	< LOD	< LOD
Brass	5.26	0.78	< LOD	< LOD	< LOD	< LOD

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

Figure 3. Test 6 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.