Test Plan for the Natural Attenuation of SARS-CoV-2 as a Decontamination Approach

Prepared For:

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ACRONYMS AND ABBREVIATIONS

Acronym or Abbreviation	Definition
ATCC	American Type Culture Collection
BBRC	Battelle Biomedical Research Center
BSCIII	Class III Biosafety Cabinet
BSL	Biosafety Level
C	Celsius
CFU	Colony Forming Units
COTR	Contract Officer's Technical Representative
CoV	Coronavirus
CPE	Cytopathic Effects
CTRL	Control
C_T	Threshold Cycle
DECON	Decontaminated
F	Fahrenheit
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
IMLS	Institute of Museum and Library Services
mL	Milliliter
NIST	National Institutes of Standards and Technology
OCLC	
PBS	Phosphate Buffered Saline
PES	Polyethersulfone
PFU	Plaque Forming Units
PI	Principal Investigator
PM	Program Manager
QA	Quality Assurance
QC	Quality Control
RG	Risk Group
RH	Relative Humidity
SARS	Severe Acute Respiratory Syndrome
SOP	Standard Operating Procedure
TBD	To Be Determined
$TCID_{50}$	Median Tissue Culture Infectious Dose
TIR	Test Incident Report
TRR	Test Readiness Review

1.0 INTRODUCTION

1.1 Background

The Institute of Museum and Library Services (IMLS) and OCLC are working 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 research collaboration will provide information on how long the virus survives on surfaces and how or if materials can be handled to mitigate exposure.

Current information is conflicting and inconclusive regarding how long the virus survives on surfaces and how – or if – materials can be handled to mitigate exposure. This concern has widespread implications as materials handling is a key function in many industries across the globe.

1.2 Objective

The overall objective of this project is to gather data for OCLC and IMLS on the efficacy of ambient environmental conditions (temperature and relative humidity [RH]) against SARS-CoV-2 pathogenic virus applied to representative materials found in libraries, archives and museums. OCLC and IMLS will select the materials and number of organisms to be tested, and Battelle will obtain and experiment with the agents.

1.3 Limitations

Severe Acute Respiratory Syndrome (SARS)-coronavirus [CoV]-2 will be used for this study, the starting titer of Battelle's SARS-CoV-2 stock is at approximately 1×10^6 median tissue culture infectious dose ($TCID_{50}$)/mL, it will not be possible to resolve or measure the ≥6-log reduction possible with high concentrations of *Bacillus anthracis* spores, for example. This is due to the limitation of virus replication within the host VERO cell in which it is propagated. Based on recent working calculations, the maximum log reduction (assuming no detectable virus from the decontaminated samples) may be approximately 3.5 logs.

Testing will not be conducted in accordance with the US Food and Drug Administration (FDA) Good Laboratory Practices (GLP) regulations 21 CFR Part 58 (*i.e.*, non-GLP) since data generated is not intended for licensure of a product with FDA or other regulatory agency. However, staff will follow standard operating procedures that are typically followed for GLP programs.

The following organizations and individuals will have key roles and responsibilities for the completion of the testing described in this test plan.

2.0 TECHNICAL APPROACH

The technical approach is divided into two phases:

- Phase 1 – Library Circulation Materials
- Phase 2 – Other Library and Museum Materials

2.1 Task 1 – Test Setup

Test setup will focus on the use of temperature and relative humidity (RH) to inactivate the SARS-CoV-2 virus. Adjustment and control of the RH parameter will be considered if the ambient conditions (22±2°C and 40±10% RH) alone do not achieve the desired inactivation rates. Data will be reported in a tabular format and will include an executive level summary of results.

The test materials (referred to as "coupons") used for this test will be sized at approximately 2 cm × 5 cm to allow the volume of virus to be distributed uniformly distributed as droplets from liquid suspension. Phase 1 test materials were provided by Columbus Metropolitan Library and will consist of a hardcover, buckram cloth book cover, plastic protective book covering, book pages (closed within book), a paperback book cover, and DVD case. To replicate real world contamination conditions, the SARS-CoV-2 virus may be mixed into synthetic saliva made per ASTM method E2721-16.

The stock material will be inspected upon arrival at the BBRC. The stock material will be cut to size for testing. They will either be washed with soap and water followed by sterilization via autoclave (121°C, 15 pounds per square inch, 30 minutes) or gently wiped with 70% ethanol disinfectant based on each material's temperature tolerance and chemical compatibility. The coupons will be tested for confirmation of sterility by extraction and plating the extractant onto cell culture monolayer (Negative Control). The material coupons will be handled in a manner to maintain sterility upon post-treatment. As a precaution, the virus extracts will be filter-sterilized with a low binding, 0.2 micron, polyethersulfone (PES; or equivalent) filter to ensure that no contaminants are inadvertently introduced to the cell culture monolayer for the assay.

2.2 Task 2 – Agent Propagation

Battelle maintains an in-house stock of SARS-CoV-2 virus that was isolated from Washington state and is readily available and can be used for this effort since they propagate, characterize, and titer many of the agents used in their studies.

RNA Virus

This study will use Vero cell monolayers in T-flasks for the propagation of RNA viruses such as SARS-CoV-2 strain USA-WA1/2020 according to Battelle SOP BBRC.X-258 and Battelle SOP BBRC.X-184. The virus harvest will occur from the cell-lysate preparation that has been clarified to remove cell debris. Verification of infectivity and quantification of the RNA virus will be achieved via plaque assay as described by Battelle SOP BBRC.X-237 or the median tissue culture infectious dose ($TCID_{50}$) assay according to BBRC SOP X-171, "Determination of the Median Tissue Culture Infectious Dose ($TCID_{50}$) for a Viral Sample." The preparation can also be characterized by PCR according to Battelle SOP No. BBRC.X-146.

2.3 Task –3 Decontamination Testing

Decontamination tests will be conducted (as shown in **Table *1***) to determine the effectiveness (log reduction) of inactivating SARS-CoV-2 inoculated on up to five materials using ambient environmental conditions (*i.e.*, temperature and relative humidity). When possible, an iterative approach will be utilized such that, if virus attenuation occurs before the end of the specified time

course, the test can be halted and later timepoints can be repurposed to fill in earlier data gaps. This method will be conducted in consultation with OCLC and is subject to sufficient funding per test.

Table 1. Number of test coupons by organism, proposed material, and exposure times

Test #	Organism	Proposed Materials*	Environmental Conditions	Exposure Time and Number/Type of Coupons for Each Material
				Test Materials
1	SARS-CoV-2	Hardcover, buckram book cover Paperback book cover Book pages (closed) Plastic protective book cover DVD case	22±2°C 40±10 RH	0 Day (T0)=5 1 Day (T1)=5 3 Day (T2)=5 4 Day (T3)=5 6 Day (T4)=5 Procedural blanks = 3
2-n		TBD	TBD TBD	T0=5 T1=5 T2=5 T3=5 Procedural blanks = 3

*Actual test materials will be determined in consultation with sponsor
T = Test materials inoculated with the organism and exposed to environmental conditions (temp and %RH)
TBD = To be determined

For each agent, the experimental matrix assumes a maximum of 100 test samples (5 replicates × 1 test × 5 material types × 1 organism × 4 contact times), and 15 blank samples (3 replicate × 1 tests × 5 material types × 1 organism × 1 blank types). Ambient environmental conditions (up to 6 total tests) will be tested with up to four different contact times per test (including time zero; serves as the control for a particular time-course). Battelle assumes that no more than two time-points will be selected for any given day of testing due to sample processing constraints.

The temperature and RH of the control and test chambers will be monitored continuously and recorded during each decontamination test with an HMT368 temperature and humidity probe Vaisala, Inc., Woburn, MA, USA; or equivalent). Temperature and RH will be controlled with a CNI-822 controller (Omega Engineering, Stamford, CT, USA; or equivalent) and will be recorded once every minute or as appropriate. Control of temperature or RH during the initial drying period will not be performed. All temperature and RH measuring devices will be verified to be within calibration.

Each agent will be applied as ten droplets that consist of 10 µL each across the coupon surface (***Figure 1***) and allowed to become visibly dried.

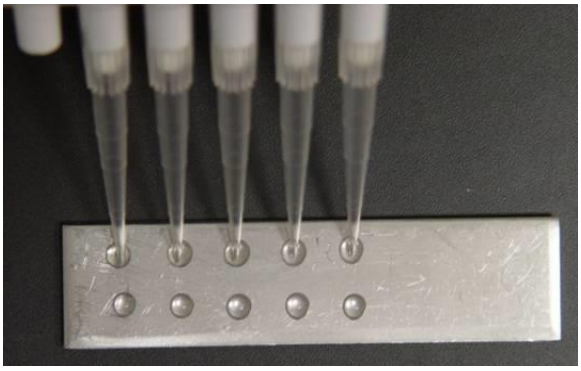

Figure 1. Liquid inoculation of coupon using a micropipette.

Five replicates of ambient environmental coupons of each material will be used during each of the up to 6 trials. The test coupons will be extracted according to Battelle Method No. 89/Microbiology, "Coupon Testing of Decontamination Formulations," as a guidance document by using 50 mL conical tubes (Fisher Scientific Cat. No. 14-959-49A, Waltham, MA, USA; or equivalent) that contain the appropriate 10 mL extraction buffer. The extraction buffer may consist of Minimum Essential Medium (MEM; Sigma Cat. No. 51416C, St. Louis, MO, USA; or equivalent) + penicillin-streptomycin (Sigma Cat. No. P4333; or equivalent) + 2 to 5% fetal bovine serum (Sigma Cat. No. F4135; or equivalent). The conical tubes will be agitated on a platform shaker at 200 rotations per minute for 15 minutes.

Potential confounding organisms will be excluded or controlled by sterilization of the coupons and use of aseptic technique. Blanks will be run in parallel with the inoculated coupons. Assuming the blanks show no indication of positive viral activity or bacterial colonies, the cytopathic effects (CPE; ***Figure 2***) from inoculated coupons will indicate viable infectious virus.

Figure 2. Cytopathic effects on a cell monolayer caused by SARS-CoV-2. Healthy cell monolayer (left) and SARS-CoV-2-infected cells (right).

Virus infectivity will be assessed by $TCID_{50}$ assay. In the $TCID_{50}$ assay, up to ten dilutions of each sample will be inoculated in quintuplicate onto a single 96-well plate at ≥70% Vero cell monolayer confluency. Viral assay plates will be incubated at 37 ± 2°C and 5 ± 2% carbon dioxide for up to 72 ± 4 hours, then observed microscopically for CPE for $TCID_{50}$ assays in triplicate wells

per dilution. Observations for CPE will be used to quantitatively calculate a viral titer for each sample.

The resulting $TCID_{50}$ values from the T0 samples will be compared against those from the inactivated samples to calculate the efficacy expressed as log reduction.

Inactivation efficacy will be calculated as the log reduction in infectious virus as a result of the ambient environmental condition. Efficacy (*E*) will be calculated for each as:

$$E = log10\ N/N'$$

where *N* is the average number of virus ($TCID_{50}$/mL) recovered from the control material coupons (*i.e.*, those inoculated, but not subjected to the condition) and *N′* as the number of virus recovered from each coupon after exposure to the environmental condition. If no infectious virus is recovered from a material after decontamination, the value 1 will be substituted for *N′*. Since the value 1 is greater than the observed value of zero, the estimated efficacy with this substitution becomes a lower bound for the true efficacy. In the case of SARS-CoV-2, the expected maximum log reduction based on the amount of virus applied and no virus detected on the inactivated coupons is approximately 3.5 logs. The test log reduction may be lower if the recovery from the control coupons is lower than the amount applied.

3.0 TRAINING, SAFETY, AND SECURITY

The biological testing included in this Test Plan requires specially trained and qualified personnel to perform the laboratory work.

Documentation of proper training is mandatory before testing takes place. Access to the test facility will be limited to Battelle staff who have met all the necessary training requirements. Battelle's PI is responsible for assuring that only properly trained and qualified personnel perform the work described in this Test Plan. All participants in this evaluation at the BBRC will adhere to the security, health, and safety requirements of the BBRC.

The existing access restrictions of the test facility will be followed. No departure from standard procedures will be needed for this evaluation. All visiting Battelle staff at the test facility will be given a site-specific safety briefing prior to the start of any test activities.

4.0 DOCUMENTATION AND RECORDS

Documentation of training related to laboratory analysis, data analysis, reporting, and project management is maintained for Battelle test personnel as required for their role/responsibilities in training files at their respective locations. The Battelle Quality Assurance (QA) Manager will verify the presence of appropriate training records.

The records for this evaluation will include the test plan, data collection forms, electronic files (both raw data and spread sheets), photographs (if needed), the draft and final reports, and QA assessment reports. All of these records will be maintained by the PI or his designee during the evaluation and transferred to permanent storage at the conclusion of the evaluation except for QA records which will be maintained by the Battelle QA Manager.

5.0 QUALITY OBJECTIVE AND PERFORMANCE CRITERIA

The quantitative assessment of decontamination efficacy at a given temperature is affected by uncertainty in several measurements: The volume of stock agent suspension inoculated onto material coupons and test suspensions; the number of viable spores, bacteria, or virus per unit volume in the stock suspension and in the coupon extracts; the test chamber temperature (ambient; not controlled during test); and the test chamber RH (ambient; not controlled during test). Critical data quality objectives for these measurements are summarized in **Table *2***.

Table 2. Critical data quality objectives

Data Required	Method	Unit	Acceptable Uncertainty in Data	Corrective Action
Application volume	Micro-pipette	mL, µL	±10%	Replace with calibrated and sufficiently accurate micropipette
Infectious virus	Manual count	$TCID_{50}$/mL	±10% (repeatability of controls)	Provide training; test assay performance; re-count questionable multi-well plates
Time	NIST-traceable clock or stopwatch	Minutes and seconds	±0.05% (2 seconds/ hour)	Replace with timepiece of comparable traceability in the event of failure; note variance in evaluation file
Temperature in test chamber	National Institute of Standards and Technology (NIST) traceable certification and/or a NIST-traceable thermometer/ hygrometer	C	±2°C	Replace with calibrated and sufficiently accurate thermometer and note variance in evaluation file
RH in test chamber	National Institute of Standards and Technology (NIST) traceable certification and/or a NIST-traceable thermometer/ hygrometer	%	±5% from 25% to 95% over the range of 5 °C to 55 °C	Replace with calibrated and sufficiently accurate hygrometer and note variance in evaluation file

If an unplanned or unanticipated incident occurs during laboratory execution, a test incident report (TIR) will be generated to detail the occurrence and the corrective action(s) required to remedy it. Battelle will immediately notify OCLC and IMLS PM, followed by submission of the TIR to OCLC and IMLS PM and Battelle's quality control auditor. All TIRs will be submitted as an appendix in the final report.