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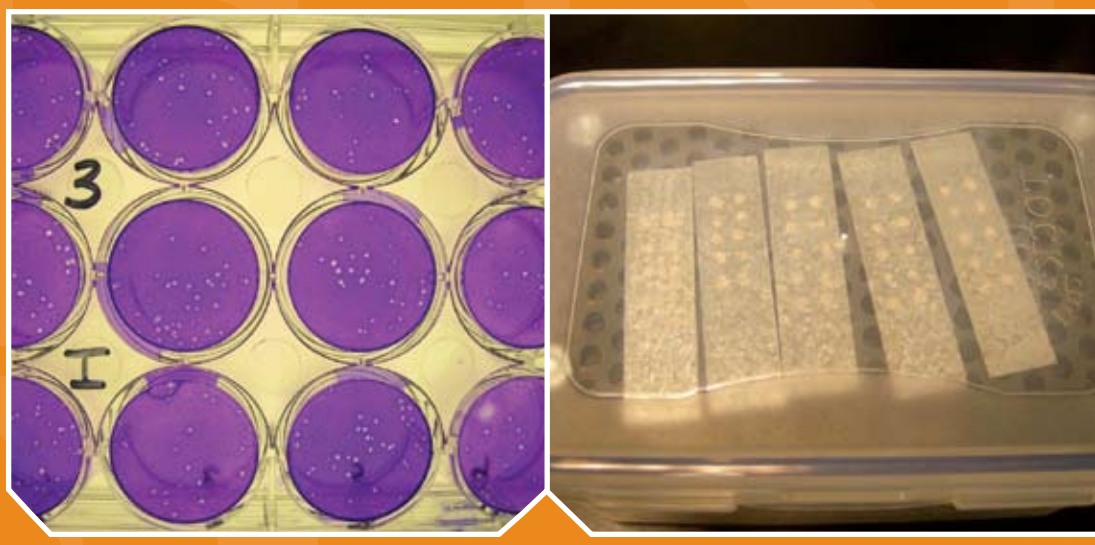
Freeze-Dried Vaccinia Virus Persistence Testing and Liquid Decontamination Technology Evaluation

INVESTIGATION AND TECHNOLOGY EVALUATION REPORT



Office of Research and Development
National Homeland Security Research Center

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INVESTIGATION AND TECHNOLOGY
EVALUATION REPORT

Freeze-Dried Vaccinia Virus Persistence Testing and Liquid Decontamination Technology Evaluation

By

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, EPA strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development (ORD) provides data and scientific support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

In September 2002, EPA announced the formation of the National Homeland Security Research Center (NHSRC). The NHSRC is part of the ORD; it manages, coordinates, and supports a variety of research and technical assistance efforts. These efforts are designed to provide appropriate, affordable, effective, and validated technologies and methods for addressing risks posed by chemical, biological, and radiological terrorist attacks. Research focuses on enhancing our ability to detect, contain, and clean up in the event of such attacks.

The NHSRC has developed the Technology Testing and Evaluation Program (TTEP) in an effort to provide reliable information regarding the performance of homeland security related technologies. TTEP provides independent, quality assured performance information that is useful to decision makers in purchasing or applying the tested technologies. It provides potential users with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies. The technology categories of interest include detection and monitoring, water treatment, air purification, decontamination, and computer modeling tools for use by those responsible for protecting buildings, drinking water supplies and infrastructure.

The evaluation reported herein was conducted by Battelle as part of TTEP. Information on NHSRC and TTEP can be found at http://www.epa.gov/nhsrc/ttep.html.

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Contents

Tables

Abbreviations/Acronyms

AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
°C	degrees Celsius
$CaCO_3$	calcium carbonate
cm	centimeter
CO_2	carbon dioxide
D/E broth	Dey and Engley broth
EPA	U.S. Environmental Protection Agency
g	gram
MDCK	Madin-Darby canine kidney
MEM	minimum essential medium
mL	milliliter
NHSRC	National Homeland Security Research Center
ORD	EPA Office of Research and Development
PBS	phosphate-buffered saline
PFU	plaque-forming unit
ppm	parts per million
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolutions per minute
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program
µL	microliter

Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) Technology Testing and Evaluation Program (TTEP) helps to protect human health and the environment from adverse impacts of terrorist acts by carrying out performance tests on homeland security technologies. Under TTEP, the persistence of viable vaccinia virus (surrogate for the variola virus, which causes smallpox) that was freeze-dried on coupons prepared from glass, galvanized metal, painted cinder block, and industrial carpet was investigated. The efficacy of two liquid decontamination technologies for inactivating the vaccinia virus that was freeze-dried on coupons of galvanized metal and industrial carpet was evaluated at room and low temperatures.

For persistence testing, materials were "contaminated" by spiking with a target level of 1×10^7 plaque-forming units (PFUs) of the vaccinia virus per coupon and immediately freeze-dried. The persistence of the vaccinia virus was subsequently investigated for up to four test durations at four environmental conditions comprised of variations in temperature and relative humidity (RH). The four environmental conditions (the four combinations of low/high RH, and low and ambient temperature) were selected for testing to investigate the effect of RH and temperature on persistence, and are as follows:

- Room temperature, low RH
  - Test durations: 14, 21, 28, and 42 days
- Room temperature, high RH
  - Test durations: 1, 3, 7, and 14 days
- Low temperature, low RH
  - Test durations: 14, 21, 28, and 56 days
- Low temperature, high RH
  - Test durations: 7, 14, 21, and 42 days

Under the "room temperature, high RH" environmental condition, the vaccinia virus remained viable for three days on glass and one day on galvanized metal and painted cinder block; the vaccinia virus was not detected on industrial carpet after one day. Under the "low temperature, high RH" environmental condition, the vaccinia virus remained viable for at least seven days on all materials.

The vaccinia virus generally remained viable longer under low RH conditions at both room and low temperatures compared to the respective tests conducted at a high RH. The vaccinia virus persisted on all four materials for at least 14 days at the "room temperature, low RH" environmental condition; the vaccinia virus persisted for 42 days on glass, galvanized metal, and painted cinder block. Under the "low temperature, low RH" environmental condition, the vaccinia virus persisted on all four materials for the 56-day duration. A summary of the actual test conditions and PFU values recovered from each of the materials is provided in Table ES-1.

The liquid decontamination technologies evaluated were 1% citric acid and hospital grade quaternary ammonium salt (732 ppm) disinfectant; these common chemicals were chosen because they are widely available for use. The evaluation was conducted at room and low temperatures with a 30-minute liquid decontaminant contact time. Both liquid decontamination technologies were applied to galvanized metal and industrial carpet coupons inoculated with the vaccinia virus and freeze-dried.

Neither 1% citric acid nor 732 ppm quaternary ammonium salt reduced the vaccinia virus to non-detectable levels. For 1% citric acid, mean log reductions in vaccinia virus PFUs on galvanized metal were 3.2 at both the room and low temperatures; on industrial carpet, log reductions were 2.6 at room temperature and 2.5 at low temperature. When 732 ppm quaternary ammonium salt was used, mean log reductions in vaccinia virus PFUs on galvanized metal were 1.5 at both the room and low temperature conditions; on industrial carpet the log reductions were less than 1.0.

Table ES-1. Summary of Persistence Test Conditions and Vaccinia Virus Recoveries[a]

Environmental Condition / Test Duration	Temperature (°C)[b]	RH (%)[b]	Mean Recovered Vaccinia Virus (PFUs)			
			Glass	Galvanized Metal	Painted Cinder Block	Industrial Carpet
Room Temperature, Low RH						
14 Days	22	10	2.43×10^{7}	1.90×10^{7}	2.57×10^{7}	4.34×10^{6}
21 Days	23	1	2.33×10^{4}	1.49×10^{4}	5.46×10^{0}	ND
28 Days	23	1	2.45×10^{1}	1.11×10^{4}	5.81×10^{3}	ND
42 Days	23	1	4.22×10^{3}	5.30×10^{3}	4.00×10^{-1} [c]	ND
Room Temperature, High RH						
1 Day	21	89	3.40×10^{0} [c]	1.66×10^{1}	2.55×10^{4}	ND
3 Days	23	93	6.93×10^{3}	ND	ND	Not Tested
7 Days	23	98	ND	ND	ND	ND
14 Days	21	89	ND	ND	ND	ND
Low Temperature, Low RH						
14 Days	7	7	6.04 x 107	2.71×10^{7}	6.27×10^{7}	1.24×10^{7}
21 Days	8[d]	8[d]	3.45×10^{6}	1.90×10^{6}	3.84×10^{6}	9.92×10^{5}
28 Days	7[e]	7[e]	4.05×10^{6}	3.04×10^{6}	3.06×10^{6}	1.07×10^{6}
56 Days	7	1	2.87×10^{4}	5.38×10^{4}	2.99×10^{4}	1.55×10^{1}
Low Temperature, High RH						
7 Days	7	90	5.22×10^{6}	1.47×10^{3} [c]	7.68×10^{6}	1.01×10^{6}
14 Days	7	101	3.26×10^{3} [c]	ND	2.54×10^{5}	1.32×10^{5}
21 Days	6[e]	95[e]	ND	ND	1.49×10^{5}	3.72×10^{4}
42 Days	7	96	ND	ND	ND	ND

[a] Vaccinia virus was applied to the coupons as an aqueous suspension, the coupons were freeze-dried, then placed in the environmental condition for the test duration. Spike amounts ranged from 6.98×10^{6} to 4.79×10^{7} PFUs.

[b] Temperature and RH values are the means based on continuous monitoring at 1-to-6-minute intervals.

[c] Vaccinia virus was not detected on some of the replicate test coupons; a value of 0 PFUs was used for non-detects in calculation of mean vaccinia virus recovery.

[d] Temperature and RH were recorded for less than one day after test initiation due to unplanned shut-down/failure of data-loggers. No unusual events occurred that would lead one to expect that the temperature and RH deviated from those of comparable tests.

[e] Temperature and RH were recorded for approximately 15 days after test initiation. No unusual events occurred that would lead one to expect that the temperature and RH deviated from those of comparable tests.

ND = Not detected; the detection limit is 10 PFUs.

1.0 Introduction

NHSRC's TTEP works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, scientists, and permitters; and with participation of individual technology developers in carrying out performance tests on homeland security technologies. In response to the needs of stakeholders, TTEP investigates the natural persistence of biological and chemical agents and evaluates the performance of innovative homeland security technologies by developing test plans, conducting evaluations, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure the generation of high quality data and defensible results. TTEP provides unbiased, third-party information supplementary to vendor-provided information that is useful to decision makers in purchasing or applying the evaluated technologies. Stakeholder involvement ensures that user needs and perspectives are incorporated into the evaluation design to produce useful performance information for each evaluated technology.

Under TTEP, persistence of viable vaccinia virus was tested after applying the vaccinia virus as an aqueous suspension to the surface of a test material which was then freeze-dried. The performance of liquid technologies for decontaminating freeze-dried vaccinia virus on various materials was also evaluated. The vaccinia virus is used in the smallpox vaccine and is a commonly used surrogate for variola virus, the etiological agent of smallpox. Variola virus is easily transportable in a temperature-stable freeze-dried state, and it is conceivable that variola virus might be aerosolized to cause infection[(1)]. The persistence of the vaccinia virus was investigated under various environmental conditions, after initially being freeze-dried on glass, galvanized metal, painted cinder block, and industrial carpet. The ability of two liquid decontamination technologies (1% citric acid and 732 ppm quaternary ammonium salt) to inactivate the vaccinia virus after the virus was initially freeze-dried on galvanized metal and industrial carpet, and subsequently held under room and low temperature conditions was also evaluated.

The persistence investigation and liquid decontamination technology evaluations were conducted according to a peer-reviewed test/QA plan[(2)] that was developed according to the requirements of the TTEP quality management plan (QMP)[(3)]. The persistence tests and decontamination evaluations used the PFU assay with Vero (African Green Monkey kidney) cells to quantify the viable vaccinia virus extracted from test materials. This report documents the log reductions in PFUs associated with a natural reduction in the viable vaccinia virus under various environmental conditions and the decrease in the viable vaccinia virus exposed to two liquid decontamination technologies.

2.0 Persistence Testing

2.1 Test Materials

Materials used for the vaccinia virus persistence testing are described in Table 2-1. Test coupons were uniformly cut to 1.9 cm width x 7.5 cm length from larger pieces of material. Edges and damaged areas were avoided in cutting test coupons. The coupons were then autoclaved or gamma-irradiated (Table 2-1) and stored under sterile conditions until use, which was intended to minimize microorganism contamination of the coupons' surfaces. All of the materials used for the vaccinia virus persistence testing have been used in previous TTEP testing.

2.2 Vaccinia Virus Recovery from Test Materials

2.2.1 Spiking the Coupons and Freeze-Drying

Each coupon was spiked with a target level of 1 x 10^7 PFUs of the vaccinia virus [American Type Culture Collection (ATCC) VR119] per coupon (i.e., 100 µL of 1 x 10^8 PFUs/mL stock suspension per coupon). Spiking of the coupons was performed using a multichannel micropipette as two rows of five droplets (10 µL per droplet) across the surface of the test coupon.

After spiking, the coupons were frozen overnight at ≤-80°C in preparation for the freeze-drying process. Using a VirTis DBT Benchtop 3.5 L Freeze-Dryer, the spiked, frozen coupons were freeze-dried until the samples were visibly dry (approximately two to four hours).

2.2.2 Vaccinia Virus Extraction and Quantification

At the completion of the freeze-drying process (time zero), the coupons were extracted and assayed for PFUs. The extraction approach involved placing each individual coupon in a 50 mL conical vial to which 10 mL of sterile extraction buffer [i.e., phosphate-buffered saline (PBS)] was added. The tubes were agitated on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature. Each coupon extract was then serially diluted in PBS (10-fold dilutions through 10^{-7}). An aliquot (0.1 mL) of the undiluted extract and each serial dilution was plated onto monolayers of Vero cells. The tissue culture plates were rocked every 15 minutes for approximately one hour at ~37°C, to allow adsorption of the vaccinia virus to the Vero cells. The cultures were then overlaid with minimum essential medium (MEM) containing 2% fetal bovine serum, 0.5% methylcellulose, and antibiotics. The cultures were incubated for 72 ± 4 hours at 37 ± 2°C in 95% air and 5% CO_2. Following incubation, crystal violet dye was added to the monolayers for 15 minutes, removed and the cells were rinsed with PBS. Plaques were visualized as clearings in the purple monolayer of Vero cells. The plaques were counted manually and the number of PFUs/mL was determined by multiplying the mean number of plaques per well by the reciprocal of the dilution. The plaque assay has a detection limit of 10 PFUs.

2.2.3 Vaccinia Virus Recovery Results

Prior to persistence testing, efforts were conducted to determine if an acceptable level of the vaccinia virus (>1% of the applied inoculum per the test/QA plan[2]) could be recovered from the coupons. The coupons were spiked and freeze-dried as described in the preceding sections. Then the vaccinia virus was extracted from the coupons and analyzed for PFUs. Initial determinations of vaccinia virus recoveries were conducted with three replicate coupons per material. As acceptable recoveries were obtained, final demonstrations using five replicate coupons were used to obtain mean

Table 2-1. Test Materials

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, width x length	Approximate Coupon Thickness	Material Preparation
Glass	C1036	Brooks Brothers	1.9 cm x 7.5 cm	0.3 cm	Autoclaved
Galvanized Metal	Industry heating, ventilation, and air conditioning standard 24 gauge galvanized steel	Accurate Fabrication	1.9 cm x 7.5 cm	0.06 cm	Cleaned with acetone; autoclaved
Painted Cinder Block (concrete)	ASTM C90. Brush and roller painted all sides. One coat Martin Senour latex primer (#71-1185) and one coat Porter Paints latex semi-gloss finish (#919)	Wellnitz	1.9 cm x 7.5 cm	0.5 cm	Autoclaved
Industrial Carpet	ShawTek, EcoTek 6 Color: mottled gray/dark brown/ black	Shaw Industries, Inc.	1.9 cm x 7.5 cm	0.7 cm	Gamma Irradiation

recovery and variance data. The vaccinia virus recovery results are provided in Table 2-2. Acceptable vaccinia virus recoveries were obtained for all materials used in persistence testing (glass, galvanized metal, painted cinder block, and industrial carpet). The percent vaccinia virus recoveries were higher when three replicates were used as compared to five replicates for glass, galvanized metal, and industrial carpet. Testing was conducted on two different days, and the higher inoculum associated with the five replicates contributed to lower percent recoveries since comparable amounts of vaccinia virus (PFUs) were recovered from the three and five replicates per material.

Mean percent vaccinia virus recoveries were calculated based on the mean of the replicate vaccinia virus percent recoveries per material and test (tests with three replicates and five replicates were conducted on different days) as:

Equation 2-1.

$$\overline{P}_{jk} = \frac{\sum_{i=1}^{n}\left[\left(\frac{P_{ijk}}{Q}\right)\times 100\,\%\right]}{n}$$

Where:

$\overline{P}_{jk}$ = mean percent vaccinia virus recovery for the j^{th} material and k^{th} test

P_{ijk} = vaccinia virus recovered for the i^{th} replicate, j^{th} material and k^{th} test (in PFUs)

Q = inoculum amount (in PFUs)

$\sum_{i=1}^{n}\left[\left(\frac{P_{ijk}}{Q}\right)\times 100\%\right]$ = sum of the replicate percent vaccinia virus recoveries based on the amount of vaccinia virus recovered (P_{ijk}) and the inoculum amount (Q)

n = number of replicate test coupons (three or five).

If no PFUs were detected for a test coupon, a value of 0 was substituted for the vaccinia virus recovery (P).

2.3 Persistence Testing Approach

The vaccinia virus was quantified from the liquid extracts obtained from the test coupons (spiked coupons placed in the exposure chamber with temperature, RH, and contact time treatments) and positive controls (spiked coupons, freeze-dried, and extracted at time zero [at the completion of freeze-drying]). Spiking and freeze-drying of the coupons and vaccinia virus extraction and quantification followed the approach described in Section 2.2.1. The log reduction in the vaccinia virus viability (quantified by determining PFUs) was calculated as $\overline{N}/N'$ where $\overline{N}$ is the mean number of PFUs from the five positive controls of a given material and environmental condition and N' is the number of PFUs from each test coupon replicate of a given material, environmental condition, and contact time. The log reduction in PFUs for each individual test coupon (R) was calculated for each of the five replicate test coupons of each material, environmental condition, and contact time as:

Equation 2-2.

$$R_{ijkl} = \log_{10}\left(\frac{\overline{N}_{jk}}{N'_{ijkl}}\right)$$

Where:

R_{ijkl} = log reduction in PFUs for the i^{th} replicate test coupon, j^{th} material, k^{th} environmental condition, and l^{th} contact time

$\overline{N}_{jk}$ = arithmetic mean PFU from the five positive controls for the j^{th} material and k^{th} environmental condition

$\overline{N'}_{ijkl}$ = PFUs recovered on the i^{th} replicate test coupon, j^{th} material, k^{th} environmental condition, and l^{th} contact time.

Table 2-2. Vaccinia Virus Recoveries

Material / Replicates	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]
Glass				
3 replicates	5.95×10^7	3/3	$3.05 \pm 0.73 \times 10^7$	85 ± 12
5 replicates	1.67×10^8	5/5	$2.37 \pm 0.16 \times 10^7$	24 ± 0.97
Galvanized Metal				
3 replicates	5.95×10^7	3/3	$0.54 \pm 2.39 \times 10^7$	90 ± 40
5 replicates	1.67×10^8	5/5	$1.90 \pm 0.66 \times 10^7$	57 ± 4.0
Painted Cinder Block				
3 replicates	5.95×10^7	3/3	$6.26 \pm 4.09 \times 10^6$	18 ± 6.9
5 replicates	1.67×10^8	5/5	$4.05 \pm 0.48 \times 10^6$	24 ± 2.9
Industrial Carpet				
3 replicates	5.95×10^7	3/3	$1.52 \pm 0.13 \times 10^7$	43 ± 2.2
5 replicates	1.67×10^8	5/5	$7.17 \pm 0.84 \times 10^6$	4.3 ± 0.50

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation.

If no PFUs were detected for a test coupon (N'), the value "1" was substituted for N'. Since the value 1 is greater than the observed value of 0, the estimate with this substitution becomes a lower bound for the true log reduction. Next, the mean log reduction in PFUs ($\overline{R}$) for the five replicate test coupons of a given material and environmental condition is calculated as:

Equation 2-3. $$\overline{R}_{jkl} = \frac{\sum_{i=1}^{n} R_{ijkl}}{n}$$

Where:

$\overline{R}_{jkl}$ = mean log reduction in PFUs for the j^{th} material, k^{th} environmental condition, and l^{th} contact time

$\sum_{i=1}^{n} R_{ijkl}$ = sum of the log reductions in PFUs for each individual test coupon (i) for the j^{th} material, k^{th} environmental condition, and l^{th} contact time

n = number of test coupon replicates (five).

The test matrix and various test conditions that were utilized for the vaccinia virus persistence testing are summarized in Table 2-3. The environmental conditions included various combinations of temperature and RH. Persistence was measured for four types of test coupons: glass, galvanized metal, painted cinder block, and industrial carpet, and the test durations ranged from 1 to 56 days. Initial time points were selected based on comparable data available in the literature (i.e., Stone et al., 2006[4]), but subsequent time points were adaptively chosen (i.e., shorter or longer durations) based on the initial test results, and tests were not conducted sequentially from the shortest to longest test duration. Please note that when possible multiple tests were initiated on the same day such that only one set of positive controls would be used per material (i.e., the same inoculum stock was used for all tests initiated on the same day).

Similar vaccinia virus (although not freeze-dried) persistence testing was previously conducted under TTEP, using galvanized metal and painted cinder block under environmental conditions of ~20°C and 40%-70% RH, and at 30°C at either high (>70%) or low RH (<40%) for up to 14 days[4]. In that study, the vaccinia virus persisted longer when exposed to low RH. The current testing was conducted at two temperatures (room and low) and two RH levels (high and low) to evaluate the influence of temperature and RH on the persistence of the vaccinia virus following the freeze-drying

Table 2-3. Persistence Tests Matrix[a]

	Temperature (°C)		RH (%)	
Target Environmental Condition / Test Duration	Mean	Range	Mean	Range
Room Temperature (22°C), Low RH (20%)				
14 Days	21.9	20.44 - 25.48	10.1	9.51 - 44.34
21 Days	22.6	21.58 - 23.47	1.0	1.00 - 39.25
28 Days	23.0	22.15 - 24.41	1.0	1.00 - 40.33
42 Days	23.1	22.42 - 24.12	1.0	1.00 - 31.57
Room Temperature (22°C), High RH (70%)				
1 Day	21.3	20.94 - 23.54	89.2	35.64 - 92.74
3 Days[a]	23.0	22.54 - 25.28	93.0	21.53 - 97.51
7 Days	22.8	21.32 - 23.33	97.9	41.50 - 100.88
14 Days	21.3	19.46 - 25.14	89.1	39.29 - 92.80
Low Temperature (10°C), Low RH (20%)				
14 Days	7.1	6.36 - 24.15	7.2	6.37 - 39.77
21 Days	7.6[b]	5.90b - 25.60[b]	7.7[b]	5.68b - 39.06[b]
28 Days	7.0[c]	6.61c - 24.87[c]	7.4[c]	6.46c - 40.51[c]
56 Days	7.0	6.64 - 24.73	1.0	1.00 - 39.02
Low Temperature (10°C), High RH (70%)				
7 Days	6.9	5.77 - 23.81	90.4	43.91 - 93.50
14 Days	7.5	7.14 - 23.81	100.6	90.39 - 102.20
21 Days	6.1[c]	5.80[c] - 25.89[c]	94.8[c]	37.11[c] - 97.34[c]
42 Days	6.6	6.31 - 23.50	95.8	46.21 - 97.38

[a] Test materials included glass, galvanized metal, painted cinder block, and industrial carpet, with the exception that industrial carpet was not tested during the 3-day duration at room temperature, high RH.

[b] Temperature and RH were recorded for less than one day after test initiation.

[c] Temperature and RH were recorded for approximately 15 days after test initiation.

process. The actual temperatures and RH levels associated with each test are provided in Table 2-3.

Test coupons were placed inside sealed Lock&Lock™ plastic storage containers. For low RH conditions, Drierite (W.A. Hammond Drierite Co.) was added to the containers, and for high RH conditions, 70% RH humidity beads (Heartfelt Industries) were added to the containers. For testing at the low temperature, the containers were placed inside a refrigerator. No special manipulations for controlling temperature were required for the room temperature testing. Temperature and RH were measured and the data recorded continuously at one-to-six-minute intervals with a HOBO U10 data logger (Onset Computer Corporation), except as noted on Table 2-3. Starting from ambient RH levels between approximately 35-45%, the RH tended to rapidly decrease during low RH tests and steadily increase during high RH tests, due to the Drierite and humidity beads, respectively. The range of RH values monitored included these relatively short acclimation periods during which the RH level (starting at ambient level) was decreasing or increasing within the storage containers. As such, occasionally the range of RH levels listed in Table 2-3 overlapped depending on the starting ambient level.

2.4 Test Results

Persistence results for each material/environmental condition combination are summarized in Tables 2-4 through 2-7. Several tests (e.g., the 21-, 28-, and 42-day tests at the "room temperature, low RH" environmental condition for glass on Table 2-4) were initiated on the same day so the same positive controls were applicable for each test. The amount of PFUs in the inoculums (as quantified by spike controls and presented as the inoculums for the positive and test coupons) ranged from 6.98×10^6 to 4.79×10^7 PFUs per coupon due to the varying amounts of the vaccinia virus titer propagated, and all calculations were based on the recorded results. A summary of vaccinia virus persistence results is provided in Table 2-8.

2.4.1 Glass

The vaccinia virus persistence results on glass are summarized in Table 2-4. With the exception of the "room temperature, high RH" environmental condition, the vaccinia virus remained viable for ≥14 days on glass. The vaccinia virus was found to remain viable on glass (with ≤3.9 mean log reductions in PFUs) for 42 days under the "room temperature, low RH" environmental condition, 56 days under the "low temperature, low RH" environmental condition, and seven days under the "low temperature, high RH" environmental condition.

The vaccinia virus was least viable on glass with high RH conditions at both room and low temperatures. For the "low temperature, high RH" environmental condition, a 0.9 mean log reduction in PFUs occurred following the 7-day duration, while durations of 14, 21, and 42 days resulted in ≥5.6 mean log reductions in PFUs. At the "room temperature, high RH" environmental condition, the vaccinia virus was viable after one and three days but with ≥4.3 mean log reductions in PFUs; the vaccinia virus was not detected at the 7- and 14-day durations. At the "low temperature, low RH" environmental condition, the virus recovery at 14 days was 126% (no outliers were identified as being outside three standard deviations of the mean). Recovery greater than 100% may be indicative of day-to-day variability within the extraction procedure and assay. Test coupons showing greater than 100% recovery or having a negative log reduction, especially at the low temperature, low RH environmental condition, are an indication of minimal inactivation of the vaccinia virus and not necessarily viral propagation or an error in measurement.

Table 2-4. Vaccinia Virus Persistence on Glass

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature, Low RH					
14 Days					
Positive Control[c]	3.72×10^7	5/5	$3.75 \pm 0.15 \times 10^7$	101 ± 4.02	-
Test Coupon[d]	3.72×10^7	5/5	$2.43 \pm 0.64 \times 10^7$	65.4 ± 17.1	0.2 ± 0.1
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
21 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$2.33 \pm 2.34 \times 10^4$	0.33 ± 0.33	2.6 ± 1.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$2.45 \pm 0.74 \times 10^1$	0.00 ± 0.00	5.0 ± 0.16
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$4.22 \pm 5.83 \times 10^3$	0.07 ± 0.09	3.9 ± 1.4
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Room Temperature, High RH					
1 Day					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	3/5	$3.40 \pm 4.82 \times 10^0$ [g]	0.00 ± 0.00[g]	6.1 ± 0.5[g]
Laboratory Blank	0	0/1	ND	_	-
Procedural Blank	0	0/1	ND	_	-
3 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$6.93 \pm 10.1 \times 10^3$	0.10 ± 0.14	4.3 ± 2.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
7 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	3.72×10^7	5/5	$3.75 \pm 0.15 \times 10^7$	101 ± 4.02	-
Test Coupon	3.72×10^7	0/5	ND[g]	0.00 ± 0.00[g]	7.6 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

Table 2-4. Vaccinia Virus Persistence on Glass (continued)

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Low Temperature, Low RH					
14 Days					
Positive Control	4.79×10^7	5/5	$3.75 \pm 0.15 \times 10^7$	78.2 ± 3.12	-
Test Coupon	4.79×10^7	5/5	$6.04 \pm 4.03 \times 10^7$	126 ± 84.2	-0.2 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$3.45 \pm 1.09 \times 10^6$	49.5 ± 15.6	-0.1 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$4.05 \pm 0.85 \times 10^6$	58.1 ± 12.1	-0.2 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
56 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	5/5	$2.87 \pm 2.21 \times 10^4$	0.41 ± 0.32	2.0 ± 0.3
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature, High RH					
7 Days					
Positive Control	4.79×10^7	5/5	$3.75 \pm 0.15 \times 10^7$	78.2 ± 3.12	-
Test Coupon	4.79×10^7	5/5	$5.22 \pm 1.96 \times 10^6$	10.9 ± 4.09	0.9 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	1/5	$3.26 \pm 7.29 \times 10^3$ [g]	0.05 ± 0.10g	5.6 ± 1.9[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	0/5	ND g	0.00 ± 0.00g	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$2.53 \pm 1.49 \times 10^6$	36.3 ± 21.4	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are inoculated, frozen overnight at ≤-80°C, freeze-dried until visibly dry (~2 to 4 hours), and then extracted at time zero (at the completion of freeze-drying).
[d] Test coupons are inoculated, freeze-dried, and exposed to the environmental condition for the test duration.
[e] Laboratory blanks are not inoculated with any virus, freeze-dried, and then extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are not inoculated with any virus, freeze-dried, and exposed to the environmental condition for the test duration.
[g] A value of 0 PFUs is used for non-detects in the calculation of recovered virus (PFUs) and % virus recovery, and a value of 1 PFU is used for non-detects in the calculation of mean log reduction.
[h] Temperature and RH were actually unknown.
ND = Not detected; the plaque assay detection limit is 10 PFUs.
"-" Not applicable.

2.4.2 Galvanized Metal

The vaccinia virus persistence results on galvanized metal are summarized in Table 2-5. Under both low RH conditions (at room and low temperature), the vaccinia virus remained viable for ≥42 days with the highest mean log reduction in PFUs of 3.9 occurring after the 42-day duration at the "room temperature, low RH" environmental condition. After 56 days in the "low temperature, low RH" environmental condition, there was a 1.7 mean log reduction in PFUs.

The vaccinia virus did not remain as viable on galvanized metal under high RH conditions (at room or low temperature) compared to the low RH conditions, with ≥5.3 mean log reductions over all test durations. The vaccinia virus was only detected after one day in the "room temperature, high RH" environmental condition and after 7 days in the "low temperature, high RH" environmental condition on galvanized metal.

Table 2-5. Vaccinia Virus Persistence on Galvanized Metal

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature, Low RH					
14 Days					
Positive Control[c]	3.72×10^7	5/5	$1.99 \pm 0.16 \times 10^7$	53.4 ± 4.36	-
Test Coupon[d]	3.72×10^7	5/5	$1.90 \pm 0.49 \times 10^7$	51.0 ± 13.2	0.0 ± 0.1
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
21 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$1.49 \pm 1.86 \times 10^4$	0.21 ± 0.27	3.4 ± 1.9
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$1.11 \pm 0.64 \times 10^4$	0.16 ± 0.09	2.7 ± 1.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$5.30 \pm 7.22 \times 10^3$	0.08 ± 0.10	3.9 ± 1.5
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Room Temperature, High RH					
1 Day					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$1.66 \pm 1.04 \times 10^1$	0.00 ± 0.00	5.3 ± 0.4
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
3 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
7 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	0/5	NDg	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					-
Positive Control	3.72×10^7	5/5	$1.99 \pm 0.16 \times 10^7$	53.4 ± 4.36	7.3 ± 0.0[g]
Test Coupon	3.72×10^7	0/5	ND[g]	0.00 ± 0.00[g]	-
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	

Table 2-5. Vaccinia Virus Persistence on Galvanized Metal (continued)

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Low Temperature, Low RH					
14 Days					
Positive Control	4.79×10^7	5/5	$1.99 \pm 0.16 \times 10^7$	41.5 ± 3.39	-
Test Coupon	4.79×10^7	5/5	$2.71 \pm 0.42 \times 10^7$	56.6 ± 8.77	-0.1 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$1.90 \pm 0.42 \times 10^6$	27.2 ± 6.02	0.1 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$3.04 \pm 0.77 \times 10^6$	43.6 ± 11.0	-0.1 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
56 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	5/5	$5.38 \pm 2.64 \times 10^4$	0.77 ± 0.38	1.7 ± 0.3
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature, High RH					
7 Days					
Positive Control	4.79×10^7	5/5	$1.99 \pm 0.16 \times 10^7$	41.5 ± 3.39	-
Test Coupon	4.79×10^7	4/5	$1.47 \pm 3.28 \times 10^3$ [g]	0.00 ± 0.01[g]	6.6 ± 1.8[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$2.34 \pm 1.09 \times 10^6$	33.6 ± 15.6	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.4 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are inoculated, frozen overnight at ≤-80°C, freeze-dried until visibly dry (~2 to 4 hours), and then extracted at time zero (at the completion of freeze-drying).
[d] Test coupons are inoculated, freeze-dried, and exposed to the environmental condition for the test duration.
[e] Laboratory blanks are not inoculated with any virus, freeze-dried, and then extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are not inoculated with any virus, freeze-dried, and exposed to the environmental condition for the test duration.
[g] A value of 0 PFUs is used for non-detects in the calculation of recovered virus (PFUs) and % virus recovery, and a value of 1 PFU is used for non-detects in the calculation of mean log reduction.
[h] Temperature and RH were actually unknown.
ND = Not detected; the plaque assay detection limit is 10 PFUs.
"-" Not applicable.

2.4.3 Painted Cinder Block

The vaccinia virus persistence results on painted cinder block are summarized in Table 2-6. The vaccinia virus persisted for at least 42 days under low RH conditions (at both room and low temperatures). At the "low temperature, low RH" environmental condition, the mean log reductions in PFUs on painted cinder block were ≤2.2 for all test durations, including the 56-day duration. The vaccinia virus was also detected from all durations tested at the "room temperature, low RH" environmental condition, but log reductions were ≥4.2 at 21, 28, and 42 days.

The vaccinia virus did not persist as long on painted cinder block under high RH conditions (room and low temperatures), compared to the respective low RH conditions. The vaccinia virus was detected after the 21-day duration, but not the 42-day duration, under the "low temperature, high RH" environmental condition. The vaccinia virus was detected only at one day for the "room temperature, high RH" environmental condition. At the "low temperature, low RH" environmental condition, the virus recovery was 131% (no outliers were identified as being outside three standard deviations of the mean). Recovery greater than 100% may be indicative of day-to-day variability within the extraction procedure and assay.

Table 2-6. Vaccinia Virus Persistence on Painted Cinder Block

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature, Low RH					
14 Days					
Positive Control[c]	3.72×10^7	5/5	$3.85 \pm 0.25 \times 10^7$	104 ± 6.66	-
Test Coupon[d]	3.72×10^7	5/5	$2.57 \pm 0.30 \times 10^7$	69.2 ± 7.94	0.2 ± 0.1
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
21 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$5.46 \pm 5.60 \times 10^0$	0.00 ± 0.00	5.9 ± 0.4
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$5.81 \pm 8.00 \times 10^3$	0.08 ± 0.11	4.2 ± 1.7
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 106$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	3/5	$4.00 \pm 1.82 \times 10^{-1}$ [g]	0.00 ± 0.00[g]	6.6 ± 0.1[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Room Temperature, High RH					
1 Day					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$2.55 \pm 1.17 \times 10^4$	0.37 ± 0.17	2.1 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
3 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.5 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
7 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.5 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	3.72×10^7	5/5	$3.85 \pm 0.25 \times 10^7$	104 ± 6.66	-
Test Coupon	3.72×10^7	0/5	ND[g]	0.00 ± 0.00[g]	7.6 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

Table 2-6. Vaccinia Virus Persistence on Painted Cinder Block (continued)

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Low Temperature, Low RH					
14 Days					
Positive Control	4.79×10^7	5/5	$3.85 \pm 0.25 \times 10^7$	80.4 ± 5.17	-
Test Coupon	4.79×10^7	5/5	$6.27 \pm 3.35 \times 10^7$	131 ± 70.0	-0.2 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$3.84 \pm 1.73 \times 10^6$	55.1 ± 24.8	-0.1 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$3.06 \pm 1.25 \times 10^6$	43.8 ± 18.0	0.0 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
56 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$2.99 \pm 2.60 \times 10^4$	0.43 ± 0.37	2.2 ± 0.4
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature, High RH					
7 Days					
Positive Control	4.79×10^7	5/5	$3.85 \pm 0.25 \times 10^7$	80.4 ± 5.17	-
Test Coupon	4.79×10^7	5/5	$7.68 \pm 9.37 \times 10^6$	16.0 ± 19.6	0.9 ± 0.5
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$2.54 \pm 1.60 \times 10^5$	3.64 ± 2.29	1.2 ± 0.5
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	-
Test Coupon	6.98×10^6	5/5	$1.49 \pm 1.17 \times 10^5$	2.13 ± 1.68	1.4 ± 0.3
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					-
Positive Control	6.98×10^6	5/5	$3.15 \pm 1.38 \times 10^6$	45.1 ± 19.8	6.5 ± 0.0[g]
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are inoculated, frozen overnight at ≤-80°C, freeze-dried until visibly dry (~2 to 4 hours), and then extracted at time zero (at the completion of freeze-drying).
[d] Test coupons are inoculated, freeze-dried, and exposed to the environmental condition for the test duration.
[e] Laboratory blanks are not inoculated with any virus, freeze-dried, and then extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are not inoculated with any virus, freeze-dried, and exposed to the environmental condition for the test duration.
[g] A value of 0 PFUs is used for non-detects in the calculation of recovered virus (PFUs) and % virus recovery, and a value of 1 PFU is used for non-detects in the calculation of mean log reduction.
[h] Temperature and RH were actually unknown.
ND = Not detected; the plaque assay detection limit is 10 PFUs.
"-" Not applicable.

2.4.4 Industrial Carpet

The vaccinia virus persistence results on industrial carpet are summarized in Table 2-7. The vaccinia virus remained viable longest under the "low temperature, low RH" environmental condition in which ≤4.9 mean log reductions in PFUs were observed after 56 days. Under the "room temperature, low RH" environmental condition, the vaccinia virus remained viable during the 14-day duration with a 0.2 mean log reduction in PFUs, but was not detected at ≥21 days.

Under high RH conditions, the vaccinia virus was not detected at room temperature after 1 day, but was viable at the low temperature. The vaccinia virus was not detected from any of the durations tested (including the shortest duration of one day) at the "room temperature, high RH" environmental condition. At the "low temperature, high RH" environmental condition, the vaccinia virus was detected at the 7-, 14-, and 21-day durations with ≤1.5 mean log reductions in PFUs; the vaccinia virus was not detected at 42 days.

Table 2-7. Vaccinia Virus Persistence on Industrial Carpet

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature, Low RH					
14 Days					
Positive Control[c]	3.72×10^7	5/5	$6.89 \pm 0.61 \times 10^6$	18.5 ± 1.65	-
Test Coupon[d]	3.72×10^7	5/5	$4.34 \pm 0.34 \times 10^6$	11.7 ± 0.92	0.2 ± 0.0
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
21 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Room Temperature, High RH					
1 Day					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
7 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	3.72×10^7	5/5	$6.89 \pm 0.61 \times 10^6$	18.5 ± 1.65	-
Test Coupon	3.72×10^7	0/5	ND[g]	0.00 ± 0.00[g]	6.8 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

Table 2-7. Vaccinia Virus Persistence on Industrial Carpet (continued)

Test Duration / Sample	Inoculum (PFUs)	PFU Detections[a]	Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Low Temperature, Low RH					
14 Days					
Positive Control	4.79×10^7	5/5	$6.89 \pm 0.61 \times 10^6$	14.4 ± 1.28	-
Test Coupon	4.79×10^7	5/5	$1.24 \pm 0.51 \times 10^7$	25.8 ± 10.6	-0.2 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	5/5	$9.92 \pm 7.96 \times 10^5$	14.2 ± 11.4	0.2 ± 0.4
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
28 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	5/5	$1.07 \pm 0.19 \times 10^5$	15.4 ± 2.74	0.0 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
56 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	5/5	$1.55 \pm 0.35 \times 10^1$	0.00 ± 0.00	4.9 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature, High RH					
7 Days					
Positive Control	4.79×10^7	5/5	$6.89 \pm 0.61 \times 10^6$	14.4 ± 1.28	-
Test Coupon	4.79×10^7	5/5	$1.01 \pm 0.40 \times 10^6$	2.11 ± 0.83	0.9 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
14 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	5/5	$1.32 \pm 0.40 \times 10^5$	1.89 ± 0.57	1.0 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
21 Days[h]					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	5/5	$3.72 \pm 2.13 \times 10^4$	0.53 ± 0.30	1.5 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
42 Days					
Positive Control	6.98×10^6	5/5	$1.13 \pm 0.27 \times 10^6$	16.2 ± 3.81	-
Test Coupon	6.98×10^6	0/5	ND[g]	0.00 ± 0.00[g]	6.1 ± 0.0[g]
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are inoculated, frozen overnight at ≤-80°C, freeze-dried until visibly dry (~2 to 4 hours), and then extracted at time zero (at the completion of freeze-drying).
[d] Test coupons are inoculated, freeze-dried, and exposed to the environmental condition for the test duration.
[e] Laboratory blanks are not inoculated with any virus, freeze-dried, and then extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are not inoculated with any virus, freeze-dried, and exposed to the environmental condition for the test duration.
[g] A value of 0 PFUs is used for non-detects in the calculation of recovered virus (PFUs) and % virus recovery, and a value of 1 PFU is used for non-detects in the calculation of mean log reduction.
[h] Temperature and RH were actually unknown.
ND = Not detected; the plaque assay detection limit is 10 PFUs.
"-" Not applicable.

2.4.5 Comparison of Persistence Testing Results

The vaccinia virus persistence results are summarized in Table 2-8 by denoting the longest duration (days) that the virus was detected and the shortest duration that the virus was not detected to bracket the length of time that the vaccinia virus remained viable after being freeze-dried on the materials and then held under the various environmental conditions. At the "room temperature, low RH" environmental condition, the vaccinia virus persisted for at least 42 days (the longest duration tested) on glass, galvanized metal, and painted cinder block, but was only detected after 14 days on industrial carpet. At the "room temperature, high RH" environmental condition, the vaccinia virus persisted three days or less on all four materials, and at the "low temperature, low RH" environmental condition the vaccinia virus persisted for at least 56 days (the longest duration tested) for all materials. Under the "low temperature, high RH" environmental condition, the vaccinia virus persisted 14 days on glass, seven days on galvanized metal, and 21 days on both painted cinder block and industrial carpet. In general, for both the room and low temperature tests, the virus remained viable longer at the lower RH.

Table 2-8. Summary of Vaccinia Virus Persistence

Material and Environmental Condition	Longest Duration (Days) Vaccinia Virus Detected	Shortest Duration (Days) Vaccinia Virus Not Detected
Glass		
Room temperature, low RH	42	NA
Room temperature, high RH	3	7
Low temperature, low RH	56	NA
Low temperature, high RH	14	21
Galvanized Metal		
Room temperature, low RH	42	NA
Room temperature, high RH	1	3
Low temperature, low RH	56	NA
Low temperature, high RH	7	14
Painted Cinder Block		
Room temperature, low RH	42	NA
Room temperature, high RH	1	3
Low temperature, low RH	56	NA
Low temperature, high RH	21	42
Industrial Carpet		
Room temperature, low RH	14	21
Room temperature, high RH	NA	1
Low temperature, low RH	56	NA
Low temperature, high RH	21	42

NA = Not available; the vaccinia virus was either detected at all durations or not detected from any duration.

3.0 Decontamination Technology Evaluation

3.1 Technology Descriptions

The liquid decontamination technologies evaluated consisted of:

- 1% Citric Acid
  - 1% citric acid was prepared by adding 1 g citric acid, anhydrous, (≥ 99.5% purity) to 99 mL hard water until completely dissolved.
- 732 ppm Quaternary Ammonium Salt
  - Hospital grade quaternary ammonium disinfectant [*n*-alkyl dimethyl benzyl ammonium chloride (6.25%), *n*-alkyl dimethyl ethylbenzyl ammonium chloride (6.25%), inert ingredients (87.5%)] was purchased from a local vendor and prepared per the vendor's guidance (3/4 ounces of disinfectant added to 1 gallon of hard-water) to obtain a solution containing 732 ppm of the quaternary ammonium active ingredient. This concentration was not independently verified in this evaluation.

All preparations and dilutions were made using Association of Official Analytical Chemists (AOAC) hard-water prepared at 400 ppm hardness as $CaCO_3$ (AOAC Official Method 960.09, *Germicidal and Detergent Sanitizing Action of Disinfectants*, Section E. *Synthetic Hard Water*, p. 11). The liquid decontamination technologies were chosen for testing because they are common chemicals used for disinfection which are readily available. All decontamination contact times were 30 minutes.

3.2 Test Matrix

Log reductions in the vaccinia virus were determined for two materials (galvanized metal and industrial carpet) following 30 minute contact with the liquid decontaminant (1% citric acid and 732 ppm quaternary ammonium salt) at room temperature (21°C) and low temperature (5°C). The experimental treatments performed are shown in Table 3-1.

The evaluation of liquid decontamination technologies utilized test coupons that were each spiked and freeze-dried as described in Section 2.2.1 for persistence testing.

For the decontamination evaluation conducted at the lower temperature, the coupons were placed in the refrigerator for one hour, before adding the decontamination liquids (which were also pre-cooled in the same refrigerator prior to use), to equilibrate the materials to the test temperature. In the decontamination evaluation spiked test coupons and control coupons were inverted (spiked surface down) and placed into separate 6 mL troughs each holding enough decontamination liquid to cover the spiked surface of the coupon. At the end of the 30-minute decontamination contact time, the coupons were removed, neutralized with 4.5 mL of Dey and Engley (D/E) broth, extracted to recover the vaccinia virus, and extracts assayed as described in Section 2.2.2. In addition, the trough solution was also analyzed to quantitate any of the vaccinia virus that washed into the trough liquid. Since the coupons were decontaminated via immersion in a liquid, RH was not a relevant parameter to include in the test matrix.

Neutralization approaches for 1% citric acid and 732 ppm quaternary ammonium salt followed the methods developed from a previous TTEP evaluation of the efficacy of 1% citric acid and 732 ppm quaternary ammonium salt against the avian influenza H5N1 virus[5]. Neutralization consisted of 75% D/E broth for both 1% citric acid and 732 ppm quaternary ammonium salt (i.e., 3 parts D/E broth to 1 part decontamination liquid). During the decontamination evaluation with the H5N1 virus, neutralized 1% citric acid and 732 ppm quaternary ammonium salt were not cytotoxic (>95% cell viability) to Madin-Darby canine kidney (MDCK) cells (which were used to quantify the H5N1 virus)[5]. Since MDCK and Vero cells are both mammalian kidney epithelial cells, it was not expected (nor subsequently observed) that the neutralized 1% citric acid or 732 ppm quaternary ammonium salt would induce cytotoxicity in Vero

Table 3-1. Decontamination Technology Evaluation Matrix

Decontamination Liquid	Material	Environmental Condition
1% Citric Acid	Galvanized metal	Room temperature (21°C)
	Galvanized metal	Low temperature (5°C)
	Industrial carpet	Room temperature (21°C)
	Industrial carpet	Low temperature (5°C)
732 ppm Quaternary Ammonium Salt	Galvanized metal	Room temperature (21°C)
	Galvanized metal	Low temperature (5°C)
	Industrial carpet	Room temperature (21°C)
	Industrial carpet	Low temperature (5°C)

cells. In addition, H5N1 virus recoveries from galvanized metal and soil exposed to neutralized 1% citric acid and 732 ppm quaternary ammonium salt were ≥2.60 x 10^5 tissue culture infectious dose of 50%, so an appreciable amount of the vaccinia virus was expected to be recovered from the neutralized decontamination technologies. In fact, the vaccinia virus has been shown to have comparable or less susceptibility to antimicrobials than influenza viruses[6-8].

3.3 Technology Evaluation

The vaccinia virus recovery and log reduction in PFUs for each decontamination liquid/environmental condition combination are summarized in Table 3-2 for galvanized metal and Table 3-3 for industrial carpet. Tables 3-2 and 3-3 also include the number of replicates for positive controls (5) test coupons (5), laboratory blanks (1), and procedural blanks (1). A summary of the log reductions obtained in all decontamination liquid tests is provided in Table 3-4. For the decontamination evaluations, the mean room temperature was 21°C with a range of 20°C to 22°C and the mean low temperature was 5°C with a range of 5°C to 6°C.

None of the decontamination technologies evaluated reduced the vaccinia virus on galvanized metal to non-detectable levels (Table 3-2). For 1% citric acid, mean log reductions in vaccinia virus PFUs on galvanized metal were 3.2 at both the room and low temperatures. When 732 ppm quaternary ammonium salt was used (at room and the low temperature), mean log reductions in vaccinia virus PFUs were 1.5 on galvanized metal.

None of the decontamination technologies evaluated reduced the vaccinia virus on industrial carpet to non-detectable levels (Table 3-3). For 1% citric acid, mean log reductions in vaccinia virus PFUs on industrial carpet were 2.6 at room temperature and 2.5 at low temperature. When 732 ppm quaternary ammonium salt was used (at room and the low temperature), mean log reductions in vaccinia virus PFUs on industrial carpet were less than 1.0.

Table 3-2. Decontamination Efficacy against Vaccinia Virus on Galvanized Metal

Decontamination Liquid / Sample	Inoculum (PFUs)	PFU Detections[a]	Mean Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature					
1% Citric Acid					
Positive Control[c]	2.24 x 10^7	5/5	2.00 ± 0.47 x 10^7	89.5 ± 20.9	-
Test Coupon[d]	2.24 x 10^7	5/5	1.23 ± 0.23 x 10^4	0.05 ± 0.01	3.2 ± 0.1
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
732 ppm Quaternary Ammonium Salt					
Positive Control	2.24 x 10^7	5/5	2.00 ± 0.47 x 10^7	89.5 ± 20.9	-
Test Coupon	2.24 x 10^7	5/5	6.16 ± 1.47 x 10^5	2.75 ± 0.66	1.5 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature					
1% Citric Acid					
Positive Control	2.60 x 10^7	5/5	2.09 ± 0.15 x 10^7	80.53 ± 5.66	-
Test Coupon	2.60 x 10^7	5/5	1.37 ± 0.15 x 10^4	0.05 ± 0.01	3.2 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
732 ppm Quaternary Ammonium Salt					
Positive Control	2.60 x 10^7	5/5	2.09 ± 0.15 x 10^7	80.53 ± 5.66	-
Test Coupon	2.60 x 10^7	5/5	7.02 ± 1.10 x 10^5	2.70 ± 0.42	1.5 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are virus-inoculated coupons that are freeze-dried, placed into troughs containing PBS (but do not undergo decontamination), exposed to the environmental condition, and extracted after the 30-minute contact time. Only one set of positive controls was used for each environmental condition and both liquid decontaminants.
[d] Test coupons are virus-inoculated coupons that are freeze-dried, placed into troughs containing the liquid decontaminant, exposed to the environmental condition, and extracted after the 30-minute contact time.
[e] Laboratory blanks are non-inoculated coupons that are freeze-dried and extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are non-inoculated coupons that are freeze-dried, placed into troughs containing the liquid decontaminant, exposed to the environmental condition, and extracted after the 30-minute contact time.
ND = Not detected; the detection limit is 10 PFUs.
"-" Not applicable.

Table 3-3. Decontamination Efficacy against Vaccinia Virus on Industrial Carpet

Decontamination Liquid / Sample	Inoculum (PFUs)	PFU Detections[a]	Mean Recovered Virus (PFUs)[b]	% Virus Recovery[b]	Mean Log Reduction[b]
Room Temperature					
1% Citric Acid					
Positive Control[c]	2.24×10^7	5/5	$5.62 \pm 0.39 \times 10^6$	25.1 ± 1.75	-
Test Coupon[d]	2.24×10^7	5/5	$1.43 \pm 0.26 \times 10^4$	0.06 ± 0.01	2.6 ± 0.1
Laboratory Blank[e]	0	0/1	ND	-	-
Procedural Blank[f]	0	0/1	ND	-	-
732 ppm Quaternary Ammonium Salt					
Positive Control	2.24×10^7	5/5	$5.62 \pm 0.39 \times 10^6$	25.1 ± 1.75	-
Test Coupon	2.24×10^7	5/5	$9.49 \pm 5.86 \times 10^5$	4.24 ± 2.62	0.8 ± 0.2
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
Low Temperature					
1% Citric Acid					
Positive Control	2.60×10^7	5/5	$5.15 \pm 0.51 \times 10^6$	19.82 ± 1.97	-
Test Coupon	2.60×10^7	5/5	$1.69 \pm 0.39 \times 10^4$	0.07 ± 0.02	2.5 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-
732 ppm Quaternary Ammonium Salt					
Positive Control	2.60×10^7	5/5	$5.15 \pm 0.51 \times 10^6$	19.82 ± 1.97	-
Test Coupon	2.60×10^7	5/5	$6.95 \pm 0.94 \times 10^5$	2.67 ± 0.36	0.9 ± 0.1
Laboratory Blank	0	0/1	ND	-	-
Procedural Blank	0	0/1	ND	-	-

[a] PFU detections: the numerator is the number of coupons with PFUs and the denominator is the number of replicate coupons.
[b] Data are expressed as mean ± standard deviation as applicable.
[c] Positive controls are virus-inoculated coupons that are freeze-dried, placed into troughs containing PBS (but do not undergo decontamination), exposed to the environmental condition, and extracted after the 30-minute contact time. Only one set of positive controls was used for each environmental condition and both liquid decontaminants.
[d] Test coupons are virus-inoculated coupons that are freeze-dried, placed into troughs containing the liquid decontaminant, exposed to the environmental condition, and extracted after the 30-minute contact time.
[e] Laboratory blanks are non-inoculated coupons that are freeze-dried and extracted at time zero (at the completion of freeze-drying).
[f] Procedural blanks are non-inoculated coupons that are freeze-dried, placed into troughs containing the liquid decontaminant, exposed to the environmental condition, and extracted after the 30-minute contact time.
ND = Not detected; the detection limit is 10 PFUs.
"-" Not applicable.

Table 3-4. Summary of Decontamination Efficacy against Vaccinia Virus

Material and Environmental Condition	Vaccinia Virus Mean Log Reduction in PFUs[a]	
	1% Citric Acid	732 ppm Quaternary Ammonium Salt
Galvanized Metal		
Room Temperature	3.2 ± 0.1	1.5 ± 0.1
Low Temperature	3.2 ± 0.1	1.5 ± 0.1
Industrial Carpet		
Room Temperature	2.6 ± 0.1	0.8 ± 0.2
Low Temperature	2.5 ± 0.1	0.9 ± 0.1

[a] Data are expressed as mean ± standard deviation.

4.0
Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the test/QA plan[2] and the TTEP QMP[3]. QA/QC procedures are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) used at the time of evaluation was verified as being certified, calibrated, or validated.

4.2 Audits

4.2.1 Performance Evaluation Audit

No performance evaluation audit was performed for biological agents and organisms because quantitative standards for these biological materials do not exist. Performance evaluation audits for analytical measurements (e.g., temperature, RH, and contact time) were found to be acceptable (allowable tolerances are from the test/QA plan[2]), as indicated in Table 4-1.

4.2.2 Technical Systems Audit

Battelle QA staff conducted a technical systems audit (TSA) on February 2, April 30, and May 4, 2009 to ensure that the evaluation was being conducted in accordance with the test/QA plan[2] and the QMP[3]. As part of the TSA, test procedures were compared to those specified in the test/QA plan, and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Task Order Leader for response. No items were noted during the course of the TSA with this evaluation. TSA records were permanently stored with the TTEP QA Manager.

4.2.3 Data Quality Audit

At least 10% of the data acquired during the persistence testing and decontamination technology evaluation were audited. A Battelle QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.3 QA/QC Reporting

Each audit (i.e., the performance evaluation audit, TSA, and data quality audit) was documented in accordance with the QMP[3] for submission to the EPA (the NHSRC QA Manager and the Task Order Project Officer). In addition, quality control samples including laboratory blanks, procedural blanks, and positive controls are reported along with the test coupon results in Sections 2 and 3 for each persistence test and decontamination evaluation conducted.

4.4 Data Review

Records and data generated from the persistence testing and decontamination technology evaluation received a QC/technical review. All data were recorded by Battelle staff. The person performing the QC/technical review was not involved in the experiments and added his/her initials and the date to a hard copy of the record being reviewed. This hard copy was returned to the Battelle staff member who stored the record.

4.5 Deviations

Deviations from the test/QA plan[2] occasionally arose once work in the laboratory was initiated. These deviations did not adversely affect data quality as described below:

1. Alternative approaches other than those proposed in the test/QA plan[2] were used for generating high RH and low temperature conditions. Specifically, instead of using a nebulizer to generate a high RH at the room temperature condition, an RH beyond 70% could be maintained by placing 70% humidity beads (Heartfelt Industries) inside an airtight Lock&Lock™ plastic storage container. Therefore the humidity beads were used. For reducing temperature, rather than running tubing from a re-circulating chiller through the glove box, testing was conducted inside a refrigerator. Target environmental conditions and acceptable measurement tolerances were often not attained with these alternative approaches as noted below.

Table 4-1. Performance Evaluation Audit Results

Measurement	Audit Procedure	Allowable Tolerance	Measured Tolerance
Temperature	Compare to independent calibrated thermometer value	±2°C	±0.2°C
RH	Compare to independent calibrated hygrometer value	±10%	±2%
Contact Time	Compare time to independent clock	2 seconds/hour	<1 second/hour

2. Temperature and RH were found difficult to control in some tests and were often outside of the allowable test measurement target ranges (±2°C for temperature and ±10% for RH) established in the test/QA plan[2], as documented in Section 2.3. However, mean temperature and RH levels indicate that the test conditions of room and low temperatures and low and high RH levels were attained. Although some of the mean test temperatures deviated from the target temperatures by a few °C, the temperature data and associated PFU data remain valid and useful. Similarly, although there were deviations from the target RH levels, the RH and PFU data remain valid.
3. Continuous monitoring of temperature and RH was not conducted during the entire vaccinia virus persistence duration for the following tests (due to an unplanned shut-down/failure of the data loggers):
   - The 21-day test at low temperature and low RH (monitoring was conducted for less than one day).
   - The 28-day test at low temperature and low RH (monitoring was conducted for approximately 15 days).
   - The 21-day test at low temperature and high RH (monitoring was conducted for approximately 15 days).

   No unusual events occurred that would lead one to expect that the temperature and RH of these tests deviated from those of comparable tests. However, we note that temperature and RH were difficult to control.
4. The target spike amount (inoculum) of the vaccinia virus (1×10^7 PFUs per coupon or 1×10^8 PFUs per mL) and the associated performance criterion of ±25% (7.5×10^6 to 1.25×10^7 PFUs per coupon), as established in the test/QA plan[2], was sometimes not attained during the method demonstration, persistence testing, and liquid decontamination evaluation. The amount of PFUs in the inoculums (as quantified by spike controls and presented as the inoculums for the positive and test coupons) ranged from 6.98×10^6 to 1.67×10^8 PFUs per coupon. These deviations did not have any adverse impacts on the testing as an appreciable amount of vaccinia virus was spiked to quantify log reductions.
5. Method development testing associated with the neutralization of the liquid decontaminants was not conducted since neutralization methods for the liquid decontaminants tested (1% citric acid and 732 ppm quaternary ammonium salt) were previously established in the companion TTEP evaluation of liquid decontamination technologies for the H5N1 virus[5].
6. Regarding the decontamination technology evaluation, the positive controls (at the 30-minute contact time) were exposed to PBS rather than being exposed only to air, which better mimicked the actual application of the decontamination liquid.

5.0 Summary

5.1 Vaccinia Virus Persistence

The persistence of the vaccinia virus was investigated on four materials (glass, galvanized metal, painted cinder block, and industrial carpet), after being inoculated onto the materials and freeze-dried, then held under four environmental conditions (comprised of room and low temperatures and low and high RH) for various durations. Under low RH conditions at both room and low temperature, the vaccinia virus persisted at least for 14 days on all materials. The vaccinia virus persisted for 42 days on glass, galvanized metal, and painted cinder block at the "room temperature, low RH" environmental condition, and the vaccinia virus persisted for 56 days on all four materials at the "low temperature, low RH" environmental condition.

The persistence of the vaccinia virus was generally reduced under high RH conditions at both room and low temperatures. Under the "room temperature, high RH" environmental condition, the vaccinia virus remained viable on glass for three days and remained viable on galvanized metal and painted cinder block for one day; the vaccinia virus was not viable on industrial carpet at the one-day duration. Under the "low temperature, high RH" environmental conditions, the vaccinia virus was detected on glass after 14 days, detected on galvanized metal after seven days, and detected on painted cinder block and industrial carpet after 21 days.

5.2 Vaccinia Virus Liquid Decontamination

The efficacy of two liquid decontamination technologies (1% citric acid and hospital grade quaternary ammonium salt disinfectant at 732 ppm) was evaluated for the vaccinia virus inoculated and freeze-dried on galvanized metal and industrial carpet. Decontamination efficacies were assessed at room and low temperatures with a 30-minute liquid decontaminant contact time.

Neither liquid decontamination technology reduced the vaccinia virus to non-detectable levels. For 1% citric acid, mean log reductions in vaccinia virus on galvanized metal were 3.2 at both the room and low temperatures; on industrial carpet log reductions were 2.6 at room temperature and 2.5 at the low temperature. When 732 ppm quaternary ammonium salt was used, mean log reductions in vaccinia virus on galvanized metal were 1.5 at both the room and low temperature conditions; on industrial carpet the log reductions were less than 1.0.

6.0
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