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United States
Environmental Protection
Agency

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Persistence Testing of *Brucella suis* on Outdoor Materials

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

Office of Research and Development
National Homeland Security Research Center, Decontamination and Consequence Management Division

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Abbreviations/Acronyms

BHI	brain heart infusion
°C	degrees Celsius
CFU	colony-forming unit(s)
cm	centimeter
EPA	U.S. Environmental Protection Agency
g	gram
mL	milliliter
NHSRC	National Homeland Security Research Center
nm	nanometer
OD	optical density
PBS	phosphate-buffered saline
QA	quality assurance
RH	relative humidity
TSA	technical systems audit
µL	microliter
UV	ultraviolet
UV-A	ultraviolet radiation within the wavelength range of 320 to 400 nanometers
UV-A/B	ultraviolet radiation within the wavelength range of 290 to 400 nanometers (i.e., UV-A and UV-B)
UV-B	ultraviolet radiation within the wavelength range of 290 to 320 nanometers
UV-C	ultraviolet radiation within the wavelength range of 180 to 290 nanometers
µW	microwatt

Executive Summary

The persistence of biological agents is influenced by environmental conditions and the materials with which these biological agents are in contact. The generation of scientifically defensible persistence data is useful for the proper planning of decontamination efficacy tests and helps formulate response or remediation plans in preparation for possible natural occurrences or intentional releases of biological agents. This report presents the results of an investigation to evaluate *Brucella suis* persistence on five materials (typically found in the outdoor environment) under various environmental conditions and exposure durations.

Persistence (recovery of viable organisms) was assessed for *B. suis* spiked onto five materials (aluminum, concrete, glass, soil, and wood). The spiked materials were then exposed to controlled environmental conditions. The environmental conditions comprised moderate temperature (about 22 °C) or low temperature (about 5 °C), and the absence or presence of ultraviolet (UV) radiation within the wavelength range of 290 to 400 nm (UV-A/B, which was intended to simulate natural sunlight). Relative humidity (RH) was ambient and not controlled. The persistence investigation lasted up to 28 days. Persistence was determined by the recovery of *B. suis* as colony-forming units (CFU) from the materials at the completion of the exposure duration.

The persistence investigation results are summarized in Table ES-1. In the absence of UV-A/B, *B. suis* persisted on aluminum, glass, and soil for at least 28 days (the longest duration tested), at both low and moderate temperatures. On concrete, *B. suis* was only recovered from the seven day test at the "low temperature, no UV" environmental condition. Because *B. suis* showed low persistence on concrete, the concrete coupons were replaced with wood coupons for the longer persistence tests. Persistence testing with *B. suis* on wood resulted in recoveries after 21 and 28 days of exposures to the "low temperature, no UV" environmental condition, but *B. suis* was not recovered from wood after 21 and 28 days of exposures to the "moderate temperature, no UV" environmental condition.

In general *B. suis* had higher CFU recoveries (persisted longer) at low temperature than at moderate temperature.

In the presence of UV-A/B, the length of time that *B. suis* persisted on aluminum and glass was reduced, so that the longest exposure durations from which *B. suis* were recovered ranged from 2 to 7 days. Such reductions in the duration of *B. suis* persistence were not as pronounced when *B. suis* was spiked onto soil and exposed to UV-A/B. In the presence of UV-A/B, *B. suis* was recovered from soil at the longest exposure durations tested (for at least 14 days at "moderate temperature, UV-A/B" and for at least 14 days at "low temperature, UV-A/B"). When exposed to UV-A/B, *B. suis* was not recovered from concrete. Testing was not conducted with *B. suis* on wood in the presence of UV-A/B.

Table ES-1. Summary of persistence test conditions and *B. suis* recoveries*

			Mean Recovered *B. suis* (CFU/coupon) by Material‡				
Duration (Days)	Temperature (°C)†	RH (%)†	Aluminum	Concrete	Glass	Soil	Wood
Moderate Temperature, No UV							
7	23	39	7.71×10^4	0	3.32×10^4	2.66×10^4	NT
14	23	29	2.29×10^4	0	7.36×10^3	9.29×10^3	NT
21	23	39	1.38×10^4	NT	6.26×10^2	6.88×10^2	ND
28	23	39	5.19×10^2	NT	7.87×10^2	1.13×10^2	ND
Moderate Temperature, UV-A/B§							
1	22	50	NT	ND	2.53×10^2	NT	NT
2	23	50	NT	ND	ND	NT	NT
4	22	50	1.80×10^2	ND	ND	5.03×10^4	NT
7	22	50	2.27×10^2	ND	ND	3.49×10^3	NT
10	22	50	ND	NT	NT	1.67×10^3	NT
14	23	48	ND	NT	NT	4.33×10^2	NT
Low Temperature, No UV							
7	5.3	52	8.75×10^6	5.32×10^1	2.23×10^6	9.67×10^4	NT
14	5.8	50	1.81×10^6	ND	2.07×10^5	5.41×10^4	NT
21	4.1	11	9.82×10^5	NT	7.75×10^5	9.35×10^4	1.73×10^5
28	4.4	12	3.48×10^5	NT	3.05×10^5	4.14×10^4	1.57×10^5
Low Temperature, UV-A/B§							
1	6.5	53	4.10×10^3	ND	4.23×10^3	4.24×10^6	NT
2	7.6	46	2.20×10^2	ND	4.21×10^2	4.05×10^6	NT
2#	4.9	58	8.68×10^1	ND	ND	4.57×10^6	NT
5	4.9	59	ND	ND	ND	1.01×10^6	NT
5#	4.5	58	1.32×10^1	NT	NT	7.69×10^5	NT
14	4.2	57	NT	NT	NT	7.11×10^3	NT

* Spike controls ranged from 5.67×10^7 to 1.22×10^8 CFU/coupon.
† Mean temperature and RH values based on continuous monitoring at 1 to 2 minute intervals.
‡ "ND" indicates that no viable organisms were recovered from any of the replicate coupons. The detection limit for a given coupon with triplicate plating is approximately 33 CFU/coupon (see Section 2.6).
§ UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
# Tests were repeated to improve the quantification of the UV measurements.
"NT" is a condition/material that was not tested.

1.0 Introduction

The U.S. Environmental Protection Agency (EPA) is investigating the persistence of biological and chemical agents in the absence of decontamination. For biological agents, persistence reflects the extent to which viability is retained over a defined period of time. Some biological agents are unstable and lose viability within minutes of their release, thereby diminishing the risk to human health and the environment; other agents can remain viable for weeks, months, or years. The persistence of biological agents is influenced by environmental conditions and the materials with which they are in contact. The generation of scientifically defensible persistence data is useful for the proper planning of decontamination efficacy tests and helps formulate response plans in preparation for possible natural occurrences or intentional releases of biological agents.

This investigation focused on the persistence of *Brucella suis*, a bacterium that can cause a debilitating influenza-like illness in humans. The intent was to determine the length of time that *B. suis* remained viable on various materials found in the outdoors (aluminum, concrete, glass, soil, and wood) under various environmental conditions, primarily while exposed to moderate or low temperatures and in the presence or absence of ultraviolet (UV) radiation levels typical of those associated with sunlight.

2.0 Investigation Approach

This report describes the investigation of the persistence of *B. suis* on various materials exposed to various temperature and UV radiation conditions. Briefly, *B. suis* was spiked onto materials (aluminum, concrete, glass, soil, and wood) that could become contaminated in the outdoor environment and then be exposed to environmental conditions for controlled exposure durations. Persistence was then assessed by quantifying the recovery of *B. suis* as colony-forming units (CFU) from each tested combination of material, environmental condition, and exposure duration. All testing was performed in accordance with the peer-reviewed and EPA-approved *Quality Assurance/Test Plan for Persistence Testing of Brucella suis on Outdoor Materials*.[1]

2.1 Biological Agent

The biological agent *B. suis* is a gram-negative, aerobic, non-spore-forming coccobacillus.[2] For this investigation, *B. suis* biotype I (Battelle BRU163) was used. The *B. suis* biotype I was originally obtained from American Type Culture Collection (Manassas, VA) and maintained in pure culture by Battelle. Fresh *B. suis* culture was prepared in advance of each day that coupons were spiked by transferring colonies from a streak plate (freshly growing or stored less than two weeks at 2 °C to 8 °C) into 10 mL of brain heart infusion (BHI) broth (BD Diagnostic Systems, Sparks, MD) and incubated overnight at 37 °C ± 2 °C on an orbital shaker set to 200 revolutions per minute, until an increase in turbidity was observed. The bacterial culture (late log phase of growth) was diluted with BHI broth to an optical density (OD) reading at 600 nm (OD_{600} nm) of approximately 0.1 to 0.2 OD units. The 0.1 to 0.2 OD_{600} nm has previously been shown to yield average CFU/mL that would meet the stock suspension spore concentration requirements for this investigation ($1 \times 10^7 - 1 \times 10^9$ CFU/mL). The viable *B. suis* bacteria in the stock suspension were enumerated to determine CFU/mL ("spike control") by analyzing serial dilutions (serial 1:10 dilutions) of the stock suspension prepared using phosphate-buffered saline (PBS) and plated onto BHI agar for CFU determination.

2.2 Test Materials

Materials typically found in the outdoors that were used for *B. suis* persistence testing are described in Table 2-1. Test coupons of the outdoor materials were generally cut to the sizes indicated in Table 2-1 from larger pieces of stock material. Concrete coupons were poured into molds rather than being cut to size. Soil "coupons" consisted of 3.5 cm diameter Petri dishes with a height of 1 cm lined with Parafilm® and filled with equal masses (7 g ± 1 g) of uncompacted soil. Coupons were sterilized by autoclaving or gamma irradiation (no pre-cleaning of the coupons occurred prior to sterilization). The selected approach, as shown in Table 2-1, was based on cost-effectiveness and minimization of physical alterations of the material. Autoclaving followed Battelle's standard operating procedure.[3] Gamma-irradiation at 40 kilogray was conducted by STERIS Isomedix Services (Libertyville, IL). Prior to gamma irradiation, coupons were sealed in 6 mL Uline® poly tubing (Uline, Chicago, IL) to preserve sterility until the coupons were ready for use. Test coupons were each visually inspected prior to being used in any experiment or test. Coupons with anomalies on the application surface were discarded and not used.

Table 2-1. Test materials

Material	Lot, Batch, or Observation	Manufacturer or Supplier Name	Coupon Size, Width x Length	Coupon Thickness	Material Preparation
Aluminum (finished)	Aluminum alloy 2024	Adept Products, Inc., West Jefferson, OH	1.9 cm x 7.5 cm	0.2 cm	Autoclave
Concrete (unpainted)	5 parts sand: 2 parts cement	Wysong Concrete, Fairfield, OH	1.9 cm x 7.6 cm	1.3 cm	Autoclave
Glass	American Society for Testing and Materials International C1036	Brooks Brothers Glass and Mirror Service, Columbus, OH	1.9 cm x 7.5 cm	0.3 cm	Autoclave
Soil (topsoil)	Batch No. PY1A0597	GardenScape, Inc., Eau Claire, PA	--*	--*	Gamma irradiation
Wood (untreated pine)	Generic molding	West Jefferson Hardware, West Jefferson, OH	1.9 cm x 7.5 cm	1 cm	Gamma irradiation

* Soil "coupons" consisted of a 3.5 cm diameter Petri dish with a height of 1 cm (or equivalent) lined with Parafilm® and filled with 7 g ± 1 g of uncompacted soil.

2.3 Spiking Coupons

The stock suspension (approximately 1 x 10^8 CFU/mL, prepared as described in Section 2.1) was used to spike the coupons. Test and positive control coupons were placed lying flat in the cabinet and spiked with a 100 µL aliquot of stock suspension of approximately 1 x 10^8 CFU/mL of *B. suis* generally using a multichannel micropipette as two rows of five droplets (10 µL per droplet) across the surface of the coupons (Figure 2-1); soil coupons were spiked using a single channel pipette to apply ten 10 µL droplets across the material surface. The 100 µL aliquot of stock suspension yielded approximately 1 x 10^7 CFU/coupon. Spiked coupons were immediately exposed to the test conditions without a drying period.

Spiking of test coupons with *B. suis* was performed in a Class III biological safety cabinet. To ensure further cleanliness and prevent contamination of test surfaces, sterile techniques following Battelle policies and guidelines[4-6] were exercised during all phases of handling the test coupons. Prior to spiking, each coupon was assigned a unique identifier code by the test personnel for traceability. The identifier code was placed on the coupons and test tubes in indelible ink.

2.4 Environmental Conditions

The four environmental conditions tested were based on temperature (moderate and low) and UV radiation (absent or present). UV radiation, within the wavelength range of 290 nm to 400 nm (UV-A/B) was intended to simulate natural sunlight. Relative humidity (RH) was ambient; efforts were not taken to achieve an especially low, high, or stable RH level during any of the tests. The four environmental conditions included:

- Moderate temperature, ambient (about 22 °C), no UV
- Moderate temperature, ambient (about 22 °C), UV-A/B (12-hour on/off cycle)
- Low temperature, about 4 °C, no UV
- Low temperature, about 7 °C, UV-A/B (12-hour on/off cycle).

The target temperature for the "low temperature, UV-A/B" environmental condition was 7 °C, rather than 4 °C which was the target temperature for the "low temperature, no UV" environmental condition. The 7 °C target temperature was selected to avoid potential freezing/thawing cycles that might have been generated with the use of heat-producing lamps inside a refrigerator.

Persistence testing was conducted inside a compact glove box (Plas Labs, Inc., Lansing, MI) for moderate temperature tests and inside a refrigerator modified with glove ports for the low temperature tests. In the low temperature testing, temperature and RH were monitored (every one to two minutes) using a Yokogawa DX2010 data logger (Yokogawa Electric Corporation, Tokyo) connected to an Omega HX93AC temperature/RH probe (Omega Engineering Inc., Stamford, CT); for moderate-temperature testing a HOBO U10 data logger (Onset Computer Corporation, Bourne, MA) was used to measure temperature and RH. Although RH was monitored, efforts to manipulate the RH level were not undertaken. The actual temperatures and RH levels observed during testing are documented in the test matrix (Table 2-3) presented in Section 2.5.

Fluorescent UV-A/B lamps inside the glove box or refrigerator were used to generate the UV-A/B (see Figure 2-2 for a photograph of the refrigerator configured with the UV-A/B lamps). UV radiation conditions were designed to approximate the intensity and wavelengths of UV radiation (especially UV-B, with UV radiation within the wavelength range of 290 to 320 nm) encountered in natural sunlight at the earth's surface. UV-B is reported to be the primary component of sunlight which inhibits biological activities.[7] The spectrum and intensity of terrestrial UV radiation is variable and is affected by time of day, day of year, geographical location, atmospheric pollution, and clouds. Naturally- occurring UV-B levels, observed around noon, range from 19.5 to 150 µW/cm^2.[8-11] The target UV-B level of 70 µW/cm^2 was generated using ReptiSunTM 10.0 Linear Fluorescent UV-B lamps (Zoo Med Laboratories, Inc., San Luis Obispo, CA). The amount of UV-A (UV radiation within the wavelength range of 320 to 400 nm) generated

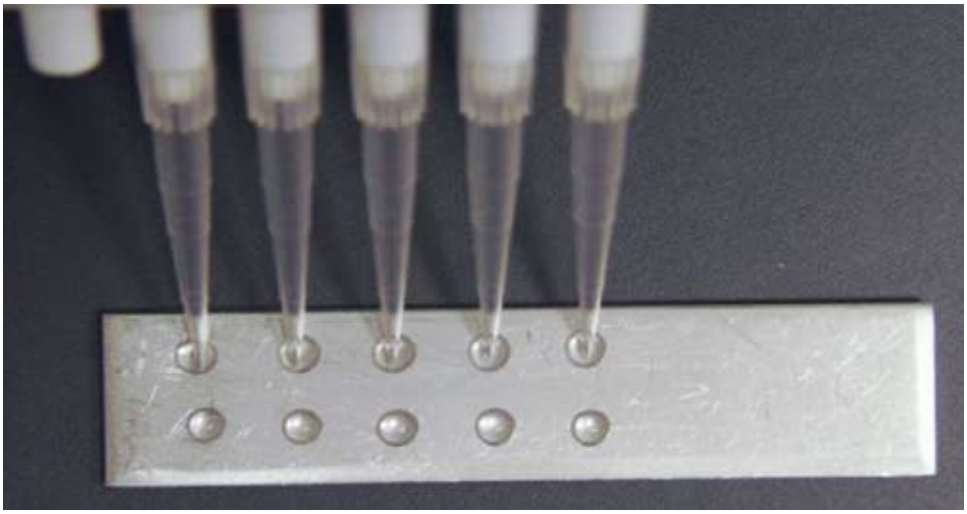


Figure 2-1. Spiking coupon using a multichannel pipette

during testing was approximately 110 μW/cm^2, which was within the range of UV-A observed in natural sunlight (0 to 4,500 μW/cm^2).[12] The level of UV-C (UV radiation within the wavelength range of 180 to 290 nm) generated during testing was 0 μW/cm^2; solar UV-C does not generally reach the earth's surface.[12, 13] UV radiation was conducted for 12 hours (UV-A/B lamps on), and then the UV-A/B lamps were turned off for 12 hours to simulate diurnal conditions.

UV radiation was measured with Solarmeter® Model 5.7 for total UV radiation, Model 6.2 for UV-B, and Model 8.0 for UV-C, from Solartech, Inc. (Harrison Township, MI). UV radiation measurements were made from five positions beneath the UV-A/B lamps (Figure 2-3). UV radiation measurements were taken beneath the UV-A/B lamps at the same distance the test coupons were from the UV-A/B lamps.

UV-B, UV-C, and total UV radiation were generally monitored twice a day (at least four hours apart) during persistence testing when the UV-A/B lamps were operating (UV-A was calculated as total UV radiation minus the UV-B and UV-C levels). UV radiation measurements were generally monitored twice daily (at least four hours apart) during the persistence testing when the UV-A/B lamps were operating. UV radiation was not measured on Saturdays, Sundays, or at night. In only one instance, testing was initiated in the afternoon and therefore only one UV radiation measurement event occurred on the first test day.

During the low temperature tests a downward drift in the UV-B measurements was discovered. This drift was evident as a decreasing trend in measured UV-B levels during the five-day test at low temperature. After the test, once the UV radiation meters were allowed to warm to room temperature, the measured irradiation inside the refrigerator showed typical UV-B levels (approximately 70 μW/cm^2). Therefore, the downward trend in measurements was attributed to the prolonged exposure of the UV radiation meters to the low temperature conditions. Subsequent low temperature tests were conducted with UV radiation measurements taken only pre- and post-test, which enabled the UV radiation meters to be stored at room temperature when not in use. Although all of the UV radiation levels measured during each test are provided in Table 2-2, as footnoted, some of the low temperature measurements are believed to be inaccurate. The mean and range calculated included all irradiation measurements taken during a given persistence test. For example, the seven-day test shown in Table 2-2 resulted in 10 measurement events with five individual measurements taken at each event; the mean and range in Table 2-2 were for all fifty measurements (10 measurement events x five individual measurements per event = 50 measurements).

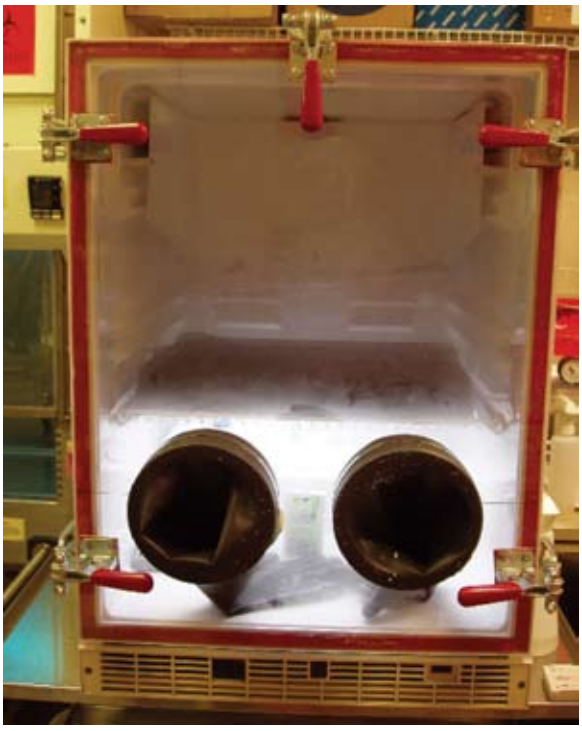


Figure 2-2. Refrigerator configured with ultraviolet-A/B lamps

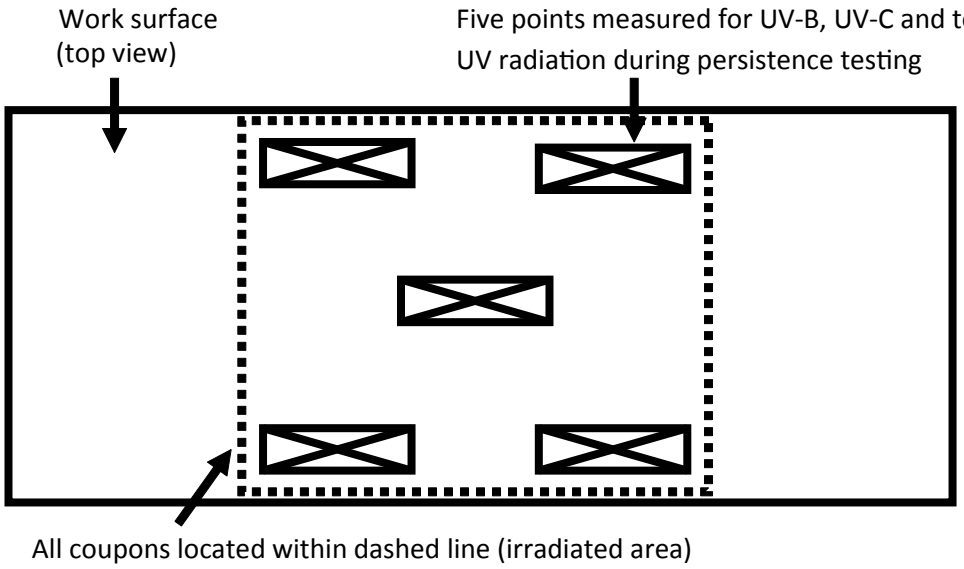


Figure 2-3. Schematic (top view) of ultraviolet radiation sampling locations (not to scale)

Table 2-2. Ultraviolet radiation levels*

Duration (Days)	UV Radiation Measurement Events	UV-A (µW/cm²)†	UV-B (µW/cm²)†
Moderate Temperature, UV-A/B‡			
1	2 (twice daily)	94 ± 7.4	68 ± 4.2
2	4 (twice daily)	94 ± 5.8	68 ± 4.4
4	4 (twice daily)	96 ± 5.5	68 ± 4.0
7	10 (twice daily)	97 ± 5.7	69 ± 4.1
10	16 (twice daily)	96 ± 5.3	68 ± 4.1
14	20 (twice daily)	96 ± 5.3	69 ± 4.0
Low Temperature, UV-A/B‡			
1	2 (pre- and post-testing)	110 ± 6.0	67 ± 5.9
2	4 (twice daily)	113 ± 5.6	63 ± 8.3§
2	2 (pre- and post-testing)	109 ± 6.9	67 ± 7.1
5	9 (twice daily)	101 ± 12	60 ± 8.8§
5	2 (pre- and post-testing)	111 ± 7.6	67 ± 5.0
14	2 (pre- and post-testing)	110 ± 6.9	66 ± 5.6

* The target UV-C level was 0 µW/cm² and all measurements were 0 µW/cm².
† Data are expressed as mean ± standard deviation.
‡ The target UV-A level was 110 µW/cm² and target UV-B level was 70 µW/cm²; UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
§ These measurements are believed to be inaccurate, due to the effect of low temperature on the meter. The actual UV-B levels are believed to be in the range of the tests in which pre- and post-testing measurements were conducted.

2.5 Test Matrix

The test matrix for persistence testing with *B. suis* is provided in Table 2-3; the order of the testing was not always done in the sequence shown in the table. After spiking the coupons, at least four non-zero exposure durations were monitored for each environmental condition. The range of exposure durations was determined in consultation with the EPA Task Order Project Officer and was based on the outcome of initial persistence test results. Positive controls (coupons spiked with *B. suis* and extracted at time-zero, i.e., immediately after spiking) and blanks (coupons not spiked with *B. suis*) were also run for each material, environmental condition, and exposure duration combination. A brief method demonstration ensured that the methods previously used[14] were adequate to obtain sufficient recovery of *B. suis* from the materials, with the exception of aluminum for which adequate recoveries were already established. Testing with wood was only occasionally conducted (i.e., in some cases *B. suis* would not persist on concrete, so persistence was tested on wood as an alternative material).

2.6 *B. suis* Recovery

For sample extraction, test coupons, positive controls, and blanks were transferred aseptically to sterile individual 50 mL conical vials containing 10 mL of sterile extraction buffer (i.e., PBS). For the soil "coupon", soil and the Parafilm liner (which prevented soil from adhering to the Petri dish) were removed from the Petri dish and placed into the vial containing the extraction buffer. The vials were agitated on an orbital shaker for 15 minutes at approximately 200 revolutions per minute at room temperature. Following extraction from the coupons, the extracts were removed and a series of dilutions (serial 1:10 dilutions) were prepared using PBS. During this investigation, *B. suis* was not recovered from any of the associated blanks.

An aliquot (0.1 mL) of the selected dilutions and, when necessary, the undiluted extract were plated onto BHI agar (BD Diagnostic Systems, Sparks, MD) in triplicate. The cultures were incubated for up to 72 hours at 37 °C ± 2 °C. The colonies were counted manually and the CFU/mL determined. Traditionally plates having colony counts between 25 and 250 are typically used for calculating the CFU/mL. However, under certain circumstances (i.e., poor recovery, reduced persistence over time, efficient decontamination, etc.) there were less than 25 colonies per plate from the undiluted extract. In these cases the number of colonies was counted and recorded even if there were 25 colonies or less per plate. The CFU/coupon were calculated by multiplying the CFU/mL by the volume of the extraction buffer used for each coupon (10 mL per coupon). The total CFU extracted from a coupon was calculated as:

Total CFU/coupon = [(mean CFU plate count x 1/dilution factor)/plated volume] x (extraction buffer volume) (1)

Where:

Mean CFU plate count	= average number of colonies counted in three replicate plates
Plated volume	= volume that was applied to each plate, in this case 0.1 mL
Dilution factor	= portion of the total extraction buffer that was used to prepare the dilutions
Extraction buffer volume	= volume of extraction buffer used to extract coupon, in this case 10 mL.

A single viable bacterium present in a plated aliquot of sample would be expected to be observed as a CFU. Considering one CFU observed on one of the three plates of undiluted extract, the individual coupon detection limit is approximately 33 CFU/coupon based on Equation 1. Since only a portion (i.e., 0.1 mL aliquot per plate) of undiluted extract is cultured, viable bacteria could be present in the

Table 2-3. *B. suis* persistence test matrix

Target Environmental Condition	Material	Duration (Days)	Temperature (°C)*	RH (%)*
Moderate temperature (22 °C), ambient RH, no UV	Aluminum, concrete‡, glass, soil, and wood§	7	22.8 ± 0.35	38.6 ± 22.5
		14	22.8 ± 0.33	29.2 ± 21.3
		21	23.3 ± 0.83	38.8 ± 2.88
		28	23.3 ± 0.83	38.8 ± 2.83
Moderate temperature (22 °C), ambient RH, UV-A/B†	Aluminum#, concrete¶, glass¶, and soil#	1	22.1 ± 0.92	49.9 ± 2.48
		2	22.6 ± 1.25	50.4 ± 4.82
		4	22.3 ± 0.95	50.1 ± 4.36
		7	22.3 ± 0.96	50.2 ± 3.94
		10	22.4 ± 1.15	49.6 ± 3.66
		14	22.7 ± 1.21	48.4 ± 4.15
Low temperature (4 °C), ambient RH, no UV	Aluminum, concrete‡, glass, soil, and wood§	7	5.29 ± 0.95	52.0 ± 15.0
		14	5.79 ± 1.00	50.0 ± 14.0
		21	4.08 ± 3.97	10.7 ± 5.87
		28	4.43 ± 3.25	11.5 ± 5.59
Low temperature (7 °C), ambient RH, UV-A/B†	Aluminum, concrete, glass, and soil⁞	1	6.47 ± 2.22	53.0 ± 24.0
		2	7.56 ± 4.30	45.5 ± 23.0
		2	4.87 ± 2.38	58.4 ± 25.7
		5	4.92 ± 2.47	58.7 ± 20.9
		5	4.48 ± 2.61	58.2 ± 25.7
		14	4.22 ± 2.94	56.6 ± 25.9

* Data are presented as mean ± standard deviation.
† The target UV-A level was 110 µW/cm² and target UV-B level was 70 µW/cm²; UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
‡ Concrete was tested only on days 7 and 14.
§ Wood was tested only on days 21 and 28.
# Aluminum and soil were tested on days 4, 7, 10, and 14.
¶ Concrete and glass were tested on days 1, 2, 4, and 7.
⁞ Only soil was tested on day 14.

extract that were not collected for plating. However, given the number of replicate coupons (five) and replicate plates (three) per undiluted coupon extract it is unlikely that the presence of viable bacteria would go undetected.

The recovery of *B. suis* bacteria (quantified as mean CFU/coupon ± standard deviation) was calculated for each material/environmental condition/exposure duration combination by dividing the total number of viable organisms extracted from all five test coupons by the number of replicate coupons (i.e., five). Persistence (recovery) curves, out to 28 days, were also developed for *B. suis* for several material and environmental condition combinations, by graphing *B. suis* recovered (quantified as CFU/coupon) against time.

3.0 Quality Assurance/Quality Control

Quality assurance (QA)/quality control procedures were performed in accordance with the program quality management plan[15] and the test/QA plan[1] for this investigation. QA/quality control procedures are summarized below.

3.1 Instrument/Equipment Testing, Inspection, and Maintenance

All equipment (e.g., pipettes, incubators, biological safety cabinets) used was verified as being certified, calibrated, or validated at the time of the investigation and was maintained and operated according to the quality requirements of the Battelle Biomedical Research Center.

3.2 Inspection/Acceptance of Supplies and Consumables

Supplies and consumables were acquired from reputable sources and were National Institute of Standards and Technology-traceable when possible. The source and purity were documented in the investigation records. Supplies and consumables were examined for evidence of tampering or damage; coupons were examined for anomalies on the test surface. Any suspect material was not used. In addition, expiration dates were noted and recorded. Solutions were prepared following Battelle Biomedical Research Center protocols and documented in reagent preparation forms.

3.3 Data Management

Data acquisition during the investigation included proper recording of the procedures used in the testing to assure consistency in the investigation and adherence to the test/QA plan[1]; documentation of sampling/testing conditions; and recording of analytical results and investigation conditions. Data acquisition was carried out electronically by the data logger (e.g., temperature, RH, and time) or manually by Battelle test personnel. Manually-acquired data were recorded immediately in a consistent format throughout the investigation. All written records were in ink and any corrections to recorded data were made with a single line through the original entry and the correction entered, initialed, and dated by the person making the correction. Non-obvious corrections included a reason for the correction. Whether collected manually or electronically, relevant data were entered into an electronic spreadsheet set up to organize the data in a clear and consistent manner. The accuracy of entering manually recorded data into the spreadsheets was checked at the time the data were entered, and a portion of the data was checked by the Battelle QA Manager as part of the data quality audit.

3.4 Assessment and Response Actions

3.4.1 Technical Systems Audit

Battelle QA staff conducted a technical systems audit (TSA) during April, May, and August 2009 to ensure that the investigation was being conducted in accordance with the test/QA plan[1] and associated amendments and the quality management plan.[15] 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. None of the findings of the TSA required corrective action. TSA records were permanently stored with the QA Manager.

3.4.2 Performance Evaluation Audit

Performance evaluation audits were performed for those measurements that factored into the data used in quantitative analysis. Table 3-1 summarizes the performance evaluation audits that were performed. The spectrophotometric absorbance was audited using a SpectraTest™ Absorbance Validation Package (MDS, Inc., Toronto, Canada) which provides a National Institute of Standards and Technology-traceable solution for validating optical performance. The test measurements and calculations are performed by SoftMax® Pro software (MDS, Inc., Toronto, Canada). No performance evaluation audit was performed for *B. suis* because quantitative standards for this biological material do not exist; however, the associated spike and positive controls and blanks form the basis of support for conclusions drawn from the investigation.

3.5 Data Quality Audit

At least 10% of the data acquired during the investigation 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.

3.6 Reports to Management

Each audit was documented in accordance with the quality management plan.[15] The results of the TSA and data quality audit were submitted to EPA.

Table 3-1. Performance evaluation audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume	Micropipettes checked by gravimetric evaluation	± 10%	Done 3 times, all <0.3%
Spectrophotometric absorbance	SpectraTest™ Absorbance Validation Package with calculations by SoftMax® Pro software	± 10% OD	Done 7 times, all <10% OD
Temperature	Compared to independent calibrated thermometer value	± 2°C	Done 4 times, all <0.4 °C
RH	Compared to independent calibrated hygrometer value	± 10% (full scale)	Done 4 times, all <1.7% (full scale)
Total UV radiation	Compared to independent calibrated total UV radiation value	± 10%	Done 4 times, all <0.6%
UV-B	Compared to independent calibrated UV-B value	± 10%	Done 4 times, all <1.4%
UV-C	Compared to independent calibrated UV-C value	± 10%	Done 4 times, all = 0.0%
Time	Compared to independent clock or watch value	2 seconds/hour	Done 4 times, all = 0 seconds/hr

3.7 Data Review

Records generated in the investigation received a quality control/technical review and a QA review before they were used to calculate, evaluate, or report investigation results. All data were recorded by Battelle staff. The person performing the review was 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.

3.8 Performance Criteria

As shown in Table 3-2, except for one stock suspension (shown in bold), all spiked amounts were within the target range of $1 \times 10^7 - 1 \times 10^9$ CFU/mL ($1 \times 10^6 - 1 \times 10^8$ CFU/coupon). One stock suspension slightly exceeded the target at 1.22×10^9 CFU/mL. The concentrations of stock suspensions ranged from $4.90 \times 10^7 - 1.22 \times 10^9$ CFU/mL.

As shown in Table 3-2 for the persistence tests, percent recoveries of viable and culturable bacteria from positive controls were within the target range for all materials except for some concrete samples (shown in bold). Although the recovery of *B. suis* from concrete during the method demonstration tests was adequate (see Table 4-1), the recovery from concrete positive controls was less than 5% for most of the subsequent persistence tests; the reason for this difference is not known. Nevertheless an appreciable amount of *B. suis* was recovered (generally $>1 \times 10^5$ CFU/coupon) from concrete to assess persistence over time.

No CFU were recovered from any blank coupons. No results were excluded as outliers.

3.9 Deviation

The test/QA plan specified that the RH was to be ambient conditions, and monitored, but not controlled. However, the data quality objectives specified in the test/QA plan set an allowable test measurement tolerance for RH in the chamber of ±20% (full scale). Because the RH range exceeded this specification, the deviation is noted. The level of RH may impact persistence, but RH was not a controlled or independent variable for this investigation. Although the RH range was higher than anticipated, mean RH levels were generally similar and ranged from 38.6% to 58.7% for all tests except three: the 14-day test at "moderate temperature, no UV" (29.2% RH) and the 21- and 28-day tests at "low temperature, no UV" (10.7% RH and 11.5% RH, respectively).

Table 3-2. Spike and positive control recovery data from persistence testing*

Material	Spike Control (CFU/Coupon)	Mean Recovered CFU/coupon from Positive Controls	Recovery as Percentage of Spike
Aluminum	6.33×10^7	$7.32 \pm 0.67 \times 10^7$	116%
	1.22×10^8	$9.99 \pm 0.78 \times 10^7$	82%
	6.27×10^7	$5.76 \pm 0.41 \times 10^7$	92%
	8.30×10^7	$9.07 \pm 1.69 \times 10^7$	109%
	5.73×10^7	$3.53 \pm 1.79 \times 10^7$	62%
	1.00×10^8	$9.42 \pm 1.99 \times 10^7$	94%
	7.07×10^7	$5.74 \pm 1.44 \times 10^7$	81%
	4.90×10^7	$4.78 \pm 0.53 \times 10^7$	98%
	7.60×10^7	$5.71 \pm 0.64 \times 10^7$	75%
	8.07×10^7	$7.27 \pm 1.22 \times 10^7$	90%
	5.67×10^7	$5.33 \pm 1.75 \times 10^7$	94%
Concrete	6.33×10^7	$9.27 \pm 20.5 \times 10^6$	15%
	8.30×10^7	$3.32 \pm 7.04 \times 10^6$	**4.0%**
	6.27×10^7	$1.91 \pm 2.64 \times 10^5$	**0.3%**
	5.73×10^7	$3.54 \pm 7.64 \times 10^6$	6.2%
	7.07×10^7	$1.81 \pm 3.49 \times 10^3$	**0.003%**
	4.90×10^7	$2.07 \pm 2.94 \times 10^5$	**0.4%**
	7.60×10^7	$1.64 \pm 2.31 \times 10^5$	**0.2%**
	8.07×10^7	$2.73 \pm 6.08 \times 10^6$	**3.4%**
Glass	6.33×10^7	$6.49 \pm 0.98 \times 10^7$	103%
	1.22×10^8	$8.65 \pm 0.49 \times 10^7$	71%
	8.30×10^7	$1.03 \pm 0.20 \times 10^8$	124%
	6.27×10^7	$6.30 \pm 0.49 \times 10^7$	100%
	5.73×10^7	$5.18 \pm 1.60 \times 10^7$	90%
	1.00×10^8	$9.86 \pm 1.80 \times 10^7$	99%
	7.07×10^7	$5.57 \pm 1.00 \times 10^7$	79%
	4.90×10^7	$6.13 \pm 0.79 \times 10^7$	125%
	7.60×10^7	$6.37 \pm 0.70 \times 10^7$	84%
	8.07×10^7	$8.33 \pm 0.75 \times 10^7$	103%
Soil	6.33×10^7	$6.09 \pm 0.65 \times 10^7$	96%
	1.22×10^8	$7.74 \pm 0.53 \times 10^7$	63%
	6.27×10^7	$5.52 \pm 0.99 \times 10^7$	88%
	8.30×10^7	$8.24 \pm 0.80 \times 10^7$	99%
	5.73×10^7	$3.02 \pm 2.03 \times 10^7$	53%
	1.00×10^8	$6.93 \pm 1.63 \times 10^7$	69%
	7.07×10^7	$4.44 \pm 1.53 \times 10^7$	63%
	7.60×10^7	$5.98 \pm 0.59 \times 10^7$	79%
	8.07×10^7	$7.67 \pm 0.69 \times 10^7$	95%
	5.67×10^7	$4.01 \pm 0.48 \times 10^7$	71%
	6.13×10^7	$4.31 \pm 0.42 \times 10^7$	70%
Wood	**1.22×10^8**	$3.60 \pm 2.70 \times 10^7$	30%
	1.00×10^8	$3.34 \pm 2.47 \times 10^7$	33%

*Spike and positive control recovery data associated with the method demonstration are presented in Table 4-1.
Bolded values are those outside of the performance criteria ranges of $1 \times 10^6 - 1 \times 10^8$ CFU/coupon based on spike control values or mean CFU ≥5% of spike levels recovered from the positive control coupons.

4.0 Test Results

For this investigation, persistence data were generated for *B. suis* in contact with up to five different materials exposed to four environmental conditions for controlled exposure durations. Persistence curves were also generated, where applicable, by graphing the *B. suis* CFU/mL derived from bacteria recovered from each material against time for each set of environmental conditions. The following sections summarize the results of the method demonstration (tests conducted initially to ensure sufficient *B. suis* recovery from each spiked material) and the persistence testing investigation.

4.1 Method Demonstration - *B. suis* Recovery

As noted in Section 2.5, a brief method demonstration was performed to ensure that previously used methods for extracting biological agents from materials were applicable for the combinations of *B. suis* and material. Results of the method demonstration are presented in Table 4-1. The *B. suis* recoveries, enumerated as CFU/coupon, attained the recovery performance criterion (mean CFU/coupon ≥5% of the spiked level) specified in the test/QA plan.[1] Recoveries of ≥5% of the bacteria spiked onto the coupon (i.e., >5.0 x 104 CFU) allow for a sufficient amount of initial bacteria to assess persistence.

4.2 Persistence Results

Persistence results for each material/environmental condition combination are summarized in Tables 4-2 through 4-6 and Figures 4-1 through 4-3. Where spike controls are identical, the coupons were spiked with the same stock suspension on the same day. An "ND" for mean recovered CFU/coupon in the result tables indicates that no CFU were detected in the undiluted extract sample plated for any of five replicate coupons. A "0" was used in the calculations of mean recovered CFU/coupon for any replicate coupon having no CFU detected. A single viable bacterium present in the plated extract would be expected to be observed as a CFU. Also note, testing at the "low temperature, UV-A/B" environmental condition was repeated for the durations of 2 and 5 days because the low temperatures appeared to adversely affect the performance of the UV meters.

4.2.1 Aluminum

The results obtained for *B. suis* persistence on aluminum are summarized in Table 4-2 and Figure 4-1. In the absence of UV-A/B, *B. suis* persisted for at least 28 days (the longest duration tested) at the "moderate temperature, no UV" and "low temperature, no UV" environmental conditions; the associated bacteria recoveries were higher at low temperature (e.g., 3.48 x 10^5 CFU/coupon at 28 days) than at moderate temperature (e.g., 5.19 x 102 CFU/coupon at 28 days).

The duration of *B. suis* persistence on aluminum decreased in the presence of UV-A/B. At the "moderate temperature, UV-A/B" environmental condition *B. suis* persisted less than 10 days, and *B. suis* did not persist or persisted at a relatively low level, i.e., 1.32 x 10^1 CFU/coupon, following the five-day duration at the "low temperature, UV-A/B" condition.

Table 4-1. Recovery data from method demonstration

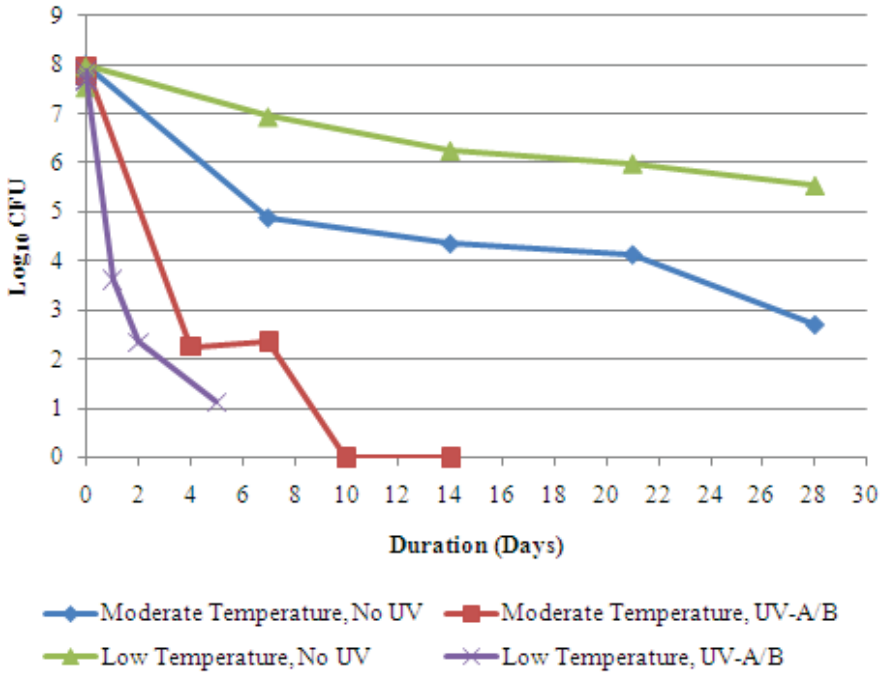
Material	*B. suis* Spike Control (CFU/coupon)	Recovered *B. suis* (CFU/coupon)*	Recovered *B. suis* (%)*
Concrete	9.33 x 10^7 (all spiked the same day with the same stock suspension)	7.98 ± 10.4 x 10^7	85.6 ± 112
Glass		4.50 ± 0.55 x 10^7	48.2 ± 5.87
Soil		4.29 ± 2.48 x 10^7	46.0 ± 26.6
Wood		2.29 ± 1.77 x 10^7	24.5 ± 18.9

* Data are expressed as mean ± standard deviation of five replicate coupons.

Table 4-2. *B. suis* persistence on aluminum

Duration (Days)*	Spike Control (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)†	
		Positive Control‡	Test Coupon§
Moderate Temperature, No UV			
7	6.33×10^7	$7.32 \pm 0.67 \times 10^7$	$7.71 \pm 3.03 \times 10^4$
14	6.33×10^7	$7.32 \pm 0.67 \times 10^7$	$2.29 \pm 1.15 \times 10^4$
21	1.22×10^8	$9.99 \pm 0.78 \times 10^7$	$1.38 \pm 1.95 \times 10^4$
28	1.22×10^8	$9.99 \pm 0.78 \times 10^7$	$5.19 \pm 4.09 \times 10^2$
Moderate Temperature, UV-A/B#			
4	6.27×10^7	$5.76 \pm 0.41 \times 10^7$	$1.80 \pm 2.06 \times 10^2$
7	6.27×10^7	$5.76 \pm 0.41 \times 10^7$	$2.27 \pm 4.88 \times 10^2$
10	8.30×10^7	$9.07 \pm 1.69 \times 10^7$	ND
14	8.30×10^7	$9.07 \pm 1.69 \times 10^7$	ND
Low Temperature, No UV			
7	5.73×10^7	$3.53 \pm 1.79 \times 10^7$	$8.75 \pm 2.89 \times 10^6$
14	5.73×10^7	$3.53 \pm 1.79 \times 10^7$	$1.81 \pm 0.49 \times 10^6$
21	1.00×10^8	$9.42 \pm 1.99 \times 10^7$	$9.82 \pm 1.18 \times 10^6$
28	1.00×10^8	$9.42 \pm 1.99 \times 10^7$	$3.48 \pm 0.57 \times 10^5$
Low Temperature, UV-A/B#			
1	7.07×10^7	$5.74 \pm 1.44 \times 10^7$	$4.10 \pm 5.60 \times 10^3$
2	4.90×10^7	$4.78 \pm 0.53 \times 10^7$	$2.20 \pm 3.31 \times 10^2$
2	7.60×10^7	$5.71 \pm 0.64 \times 10^7$	$8.68 \pm 12.4 \times 10^1$
5	8.07×10^7	$7.27 \pm 1.22 \times 10^7$	ND
5	5.67×10^7	$5.33 \pm 1.75 \times 10^7$	$1.32 \pm 1.81 \times 10^1$

* Durations were determined in consultation with the EPA Task Order Project Officer and were based on the outcome of initial persistence test results; the order of testing was not always conducted sequentially.
† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons are spiked and extracted at time zero (i.e., immediately after spiking); one set of positive controls was used for test durations initiated at the same time.
§ Test coupons are spiked and exposed to the environmental condition for the exposure duration.
# UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
"ND" indicates that no viable organisms were recovered from any of the replicate coupons. The detection limit for a given coupon with triplicate plating is approximately 33 CFU/coupon (see Section 2.6).



Notes:
Only the highest persistence results are graphed for the replicate testing on days two and five for "Low Temperature, UV-A/B".
* No CFU were detected on days 10 and 14 for "Moderate Temperature, UV-A/B".

Figure 4-1. *B. suis* persistence on aluminum

4.2.2 Concrete

The results obtained for persistence of *B. suis* on concrete are summarized in Table 4-3. On concrete, *B. suis* persisted only at the "low temperature, no UV" environmental condition, and, at that condition, *B. suis* was recovered (mean of 5.32 x 10^1 CFU/coupon) only from the seven-day exposure duration.

4.2.3 Glass

The results obtained for persistence of *B. suis* on glass are summarized in Table 4-4 and Figure 4-2. In the absence of UV-A/B, *B. suis* persisted for at least 28 days (the longest duration tested) at the "moderate temperature, no UV" and "low temperature, no UV" environmental conditions; the associated bacteria recoveries were higher at low temperature (e.g., 3.05 x 10^5 CFU/coupon at 28 days) than at moderate temperature (e.g., 7.87 x 10^2 CFU/coupon at 28 days).

The duration of *B. suis* persistence on glass decreased in the presence of UV-A/B. At the "moderate temperature, UV-A/B" environmental condition *B. suis* persisted on glass less than 2 days, and *B. suis* persisted less than 5 days at the "low temperature, UV-A/B" environmental condition.

Table 4-3. *B. suis* persistence on concrete

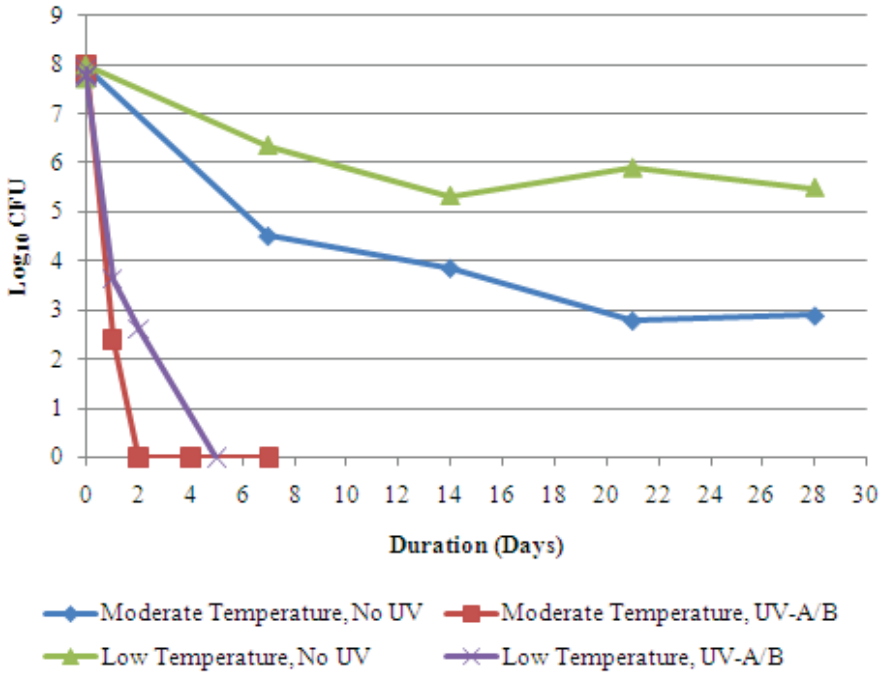
Duration (Days)*	Spike Control (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)†	
		Positive Control‡	Test Coupon§
Moderate Temperature, No UV			
7	6.33×10^7	$9.27 \pm 20.5 \times 10^6$	ND
14	6.33×10^7	$9.27 \pm 20.5 \times 10^6$	ND
Moderate Temperature, UV-A/B#			
1	8.30×10^7	$3.32 \pm 7.04 \times 10^6$	ND
2	8.30×10^7	$3.32 \pm 7.04 \times 10^6$	ND
4	6.27×10^7	$1.91 \pm 2.64 \times 10^5$	ND
7	6.27×10^7	$1.91 \pm 2.64 \times 10^5$	ND
Low Temperature, No UV			
7	5.73×10^7	$3.54 \pm 7.64 \times 10^6$	$5.32 \pm 10.2 \times 10^1$
14	5.73×10^7	$3.54 \pm 7.64 \times 10^6$	ND
Low Temperature, UV-A/B#			
1	7.07×10^7	$1.81 \pm 3.49 \times 10^3$	ND
2	4.90×10^7	$2.07 \pm 2.94 \times 10^5$	ND
2	7.60×10^7	$1.64 \pm 2.31 \times 10^5$	ND
5	8.07×10^7	$2.73 \pm 6.08 \times 10^6$	ND

* Durations were determined in consultation with the EPA Task Order Project Officer and were based on the outcome of initial persistence test results; the order of testing was not always conducted sequentially.
† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons were spiked and extracted at time zero (i.e., immediately after spiking); one set of positive controls was used for test durations initiated at the same time.
§ Test coupons were spiked and exposed to the environmental condition for the exposure duration.
# UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
"ND" indicates that no viable organisms were recovered from any of the replicate coupons. The detection limit for a given coupon with triplicate plating is approximately 33 CFU/coupon (see Section 2.6).

Table 4-4. *B. suis* persistence on glass

Duration (Days)[*]	Spike Control (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)[†]	
		Positive Control[‡]	Test Coupon[§]
Moderate Temperature, No UV			
7	6.33×10^7	$6.49 \pm 0.98 \times 10^7$	$3.32 \pm 2.37 \times 10^4$
14	6.33×10^7	$6.49 \pm 0.98 \times 10^7$	$7.36 \pm 14.0 \times 10^3$
21	1.22×10^8	$8.65 \pm 0.49 \times 10^7$	$6.26 \pm 2.99 \times 10^2$
28	1.22×10^8	$8.65 \pm 0.49 \times 10^7$	$7.87 \pm 11.2 \times 10^2$
Moderate Temperature, UV-A/B[#]			
1	8.30×10^7	$1.03 \pm 0.20 \times 10^8$	$2.53 \pm 3.04 \times 10^2$
2	8.30×10^7	$1.03 \pm 0.20 \times 10^8$	ND
4	6.27×10^7	$6.30 \pm 0.49 \times 10^7$	ND
7	6.27×10^7	$6.30 \pm 0.49 \times 10^7$	ND
Low Temperature, No UV			
7	5.73×10^7	$5.18 \pm 1.60 \times 10^7$	$2.23 \pm 1.22 \times 10^6$
14	5.73×10^7	$5.18 \pm 1.60 \times 10^7$	$2.07 \pm 0.59 \times 10^5$
21	1.00×10^8	$9.86 \pm 1.80 \times 10^7$	$7.75 \pm 3.57 \times 10^5$
28	1.00×10^8	$9.86 \pm 1.80 \times 10^7$	$3.05 \pm 1.27 \times 10^5$
Low Temperature, UV-A/B[#]			
1	7.07×10^7	$5.57 \pm 1.00 \times 10^7$	$4.23 \pm 9.16 \times 10^3$
2	4.90×10^7	$6.13 \pm 0.79 \times 10^7$	$4.21 \pm 9.22 \times 10^2$
2	7.60×10^7	$6.37 \pm 0.70 \times 10^7$	ND
5	8.07×10^7	$8.33 \pm 0.75 \times 10^7$	ND

[*] Durations were determined in consultation with the EPA Task Order Project Officer and were based on the outcome of initial persistence test results; the order of testing was not always conducted sequentially.
[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were spiked and extracted at time-zero (i.e., immediately after spiking); one set of positive controls was used for test durations initiated at the same time.
[§] Test coupons were spiked and exposed to the environmental condition for the exposure duration.
[#] UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.
"ND" indicates that no viable organisms were recovered from any of the replicate coupons. The detection limit for a given coupon with triplicate plating is approximately 33 CFU/coupon (see Section 2.6).



Notes: Only the highest persistence results are graphed for the replicate testing on day two for "Low Temperature, UV-A/B".
* No CFU were detected on days two, four, and seven for "Moderate Temperature, UV-A/B" or on day five for "Low Temperature, UV-A/B".

Figure 4-2. *B. suis* persistence on glass

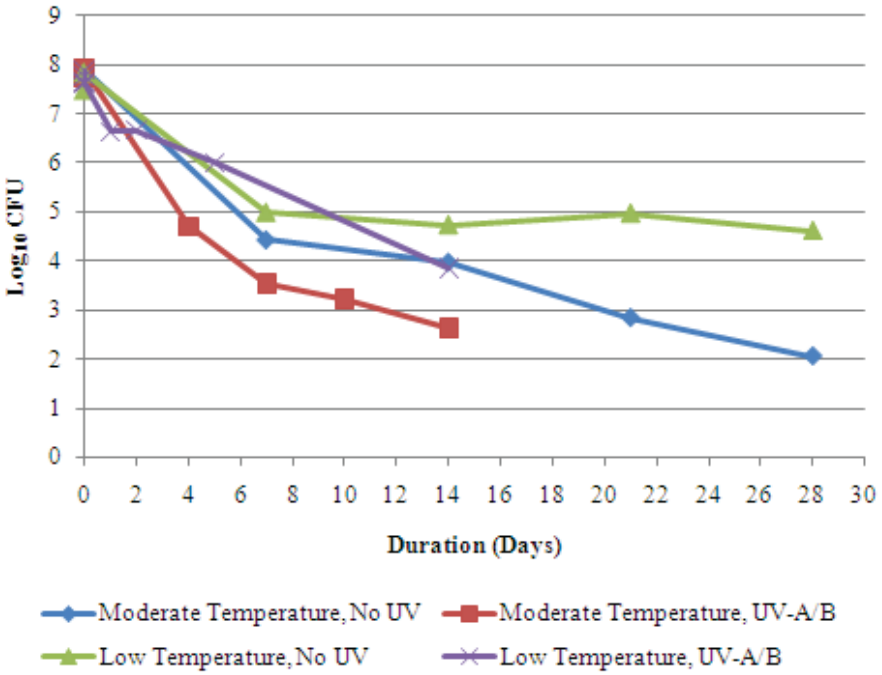
4.2.4 Soil

The results obtained for persistence of *B. suis* on soil are summarized in Table 4-5 and Figure 4-3. On soil, *B. suis* persisted under all environmental conditions for the longest durations tested: 28 days at the "moderate temperature, no UV" and "low temperature, no UV" environmental conditions, 7 days at the "moderate temperature, UV-A/B" environmental condition, and 14 days at the "low temperature, UV-A/B" environmental condition.

Table 4-5. *B. suis* persistence on soil

Duration (Days)*	Spike Control (CFU/coupon)	Mean Recovered B. suis (CFU/coupon)†	
		Positive Control‡	Test Coupon§
Moderate Temperature, No UV			
7	6.33×10^7	$6.09 \pm 0.65 \times 10^7$	$2.66 \pm 1.46 \times 10^4$
14	6.33×10^7	$6.09 \pm 0.65 \times 10^7$	$9.29 \pm 14.2 \times 10^3$
21	1.22×10^8	$7.74 \pm 0.53 \times 10^7$	$6.88 \pm 3.78 \times 102$
28	1.22×10^8	$7.74 \pm 0.53 \times 10^7$	$1.13 \pm 0.90 \times 10^2$
Moderate Temperature, UV-A/B#			
4	6.27×10^7	$5.52 \pm 0.99 \times 10^7$	$5.03 \pm 4.36 \times 10^4$
7	6.27×10^7	$5.52 \pm 0.99 \times 10^7$	$3.49 \pm 1.14 \times 10^3$
10	8.30×10^7	$8.24 \pm 0.80 \times 10^7$	$1.67 \pm 1.08 \times 10^3$
14	8.30×10^7	$8.24 \pm 0.80 \times 10^7$	$4.33 \pm 1.05 \times 10^2$
Low Temperature, No UV			
7	5.73×10^7	$3.02 \pm 2.03 \times 10^7$	$9.67 \pm 1.28 \times 10^4$
14	5.73×10^7	$3.02 \pm 2.03 \times 10^7$	$5.41 \pm 1.72 \times 10^4$
21	1.00×10^8	$6.93 \pm 1.63 \times 10^7$	$9.35 \pm 2.09 \times 10^4$
28	1.00×10^8	$6.93 \pm 1.63 \times 10^7$	$4.14 \pm 1.29 \times 10^4$
Low Temperature, UV-A/B#			
1	7.07×10^7	$4.44 \pm 1.53 \times 10^7$	$4.24 \pm 1.28 \times 10^6$
2	4.90×10^7	$4.57 \pm 0.35 \times 10^7$	$4.05 \pm 2.12 \times 10^6$
2	7.60×10^7	$5.98 \pm 0.59 \times 10^7$	$4.57 \pm 1.28 \times 10^6$
5	8.07×10^7	$7.67 \pm 0.69 \times 10^7$	$1.01 \pm 0.46 \times 10^6$
5	5.67×10^7	$4.01 \pm 0.48 \times 10^7$	$7.69 \pm 1.01 \times 10^5$
14	6.13×10^7	$4.31 \pm 0.42 \times 10^7$	$7.11 \pm 1.05 \times 10^3$

* Durations were determined in consultation with the EPA Task Order Project Officer and were based on the outcome of initial persistence test results; the order of testing was not always conducted sequentially.
† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons were spiked and extracted at time-zero (i.e., immediately after spiking); one set of positive controls was used for test durations initiated at the same time.
§ Test coupons were spiked and exposed to the environmental condition for the exposure duration.
# UV-A/B exposures were cyclical (12 hours on, 12 hours off) to simulate diurnal conditions.



Note:
Only the highest persistence results are graphed for the replicate testing on days two and five for "Low Temperature, UV-A/B".

Figure 4-3. *B. suis* persistence on soil

4.2.5 Wood

The results obtained for persistence of *B. suis* on wood are summarized in Table 4-6. Persistence testing of *B. suis* on wood was conducted only occasionally. Wood coupons were used in place of the concrete coupons in the longer persistence tests because of the lack of persistence of *B. suis* on concrete at the shorter time points. At the "moderate temperature, no UV" environmental condition, *B. suis* was not recovered from wood after a 21-day exposure duration (the shortest duration tested), but *B. suis* was recovered after 28 days of exposure to the "low temperature, no UV" environmental condition.

Table 4-6. *B. suis* persistence on wood

Duration (Days)*	Spike Control (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)†	
		Positive Control‡	Test Coupon§
Moderate Temperature, No UV			
21	1.22×10^8	$3.60 \pm 2.70 \times 10^7$	ND
28	1.22×10^8	$3.60 \pm 2.70 \times 10^7$	ND
Low Temperature, No UV			
21	1.00×10^8	$3.34 \pm 2.47 \times 10^7$	$1.73 \pm 1.43 \times 10^5$
28	1.00×10^8	$3.34 \pm 2.47 \times 10^7$	$1.57 \pm 1.22 \times 10^5$

* Durations were determined in consultation with the EPA Task Order Project Officer and were based on the outcome of initial persistence test results; the order of testing was not always conducted sequentially.
† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons were spiked and extracted at time-zero (i.e., immediately after spiking); one set of positive controls was used for test durations initiated at the same time.
§ Test coupons were spiked and exposed to the environmental condition for the exposure duration.
"ND" indicates that no viable organisms were recovered from any of the replicate coupons. The detection limit for a given coupon with triplicate plating is approximately 33 CFU/coupon (see Section 2.6).

5.0 Summary

The *B. suis* persistence results are summarized in Table 5-1. These data denote the longest exposure duration (days) that *B. suis* was recovered (persisted) and the shortest exposure duration that *B. suis* was not recovered to bracket the length of time that *B. suis* remained viable for each material and environmental condition. The shortest duration without *B. suis* recovery provides, where possible, an upper bound on the persistence of *B. suis* per materials and environmental conditions tested. In general, greater *B. suis* persistence occurred at lower temperatures (especially without exposure to UV), and *B. suis* persisted longer without exposure to simulated sunlight (although UV had less of an effect on the persistence of *B. suis* on soil).

On aluminum, glass, and soil, *B. suis* persisted for at least 28 days (the longest duration tested) under the "moderate temperature, no UV" and the "low temperature, no UV" environmental conditions. On concrete, *B. suis* did not persist following a 7-day exposure duration (the shortest duration tested) at the "moderate temperature, no UV" environmental condition, but *B. suis* on concrete did persist 7 days when exposed to the "low temperature, no UV" environmental condition. On wood, *B. suis* did not persist following a 21-day exposure duration (the shortest duration tested) at the "moderate temperature, no UV" environmental condition, but *B. suis* did persist on wood for 28 days when exposed to the "low temperature, no UV" environmental condition.

The incorporation of UV-A/B into the environmental conditions shortened the duration that *B. suis* persisted on aluminum and glass, but had less of an effect on soil. At the "moderate temperature, UV-A/B" environmental condition, the longest exposure durations associated with recovered *B. suis* were 7 days for aluminum and one day for glass. At the "low temperature, UV-A/B" environmental condition, the longest durations from which *B. suis* was recovered were 2 days on glass and 5 days on aluminum.

When exposed to UV-A/B, B. suis persisted on soil for 14 days (the longest duration tested) at the "moderate temperature, UV-A/B" environmental condition, and *B. suis* persisted on soil for 14 days (the longest duration tested) at the "low temperature, UV-A/B" environmental condition. On concrete, *B. suis* was not recovered at any of the environmental conditions that incorporated UV-A/B. Persistence testing with *B. suis* on wood was not conducted in the presence of UV-A/B.

Table 5-1. Summary of *B. suis* persistence

Material and Environmental Condition	Longest Duration (Days) with *B. suis* Recovery	Shortest Duration (Days) without *B. suis* Recovery
Aluminum		
Moderate temperature, No UV	28	AD
Moderate temperature, UV-A/B	7	10
Low temperature, No UV	28	AD
Low temperature, UV-A/B	5*	5*
Concrete		
Moderate temperature, No UV	ND	7
Moderate temperature, UV-A/B	ND	1
Low temperature, No UV	7	14
Low temperature, UV-A/B	ND	1
Glass		
Moderate temperature, No UV	28	AD
Moderate temperature, UV-A/B	1	2
Low temperature, No UV	28	AD
Low temperature, UV-A/B	2†	2†
Soil		
Moderate temperature, No UV	28	AD
Moderate temperature, UV-A/B	14	AD
Low temperature, No UV	28	AD
Low temperature, UV-A/B	14	AD
Wood		
Moderate temperature, No UV	ND	21
Moderate temperature, UV-A/B	Not tested	Not tested
Low temperature, No UV	28	AD
Low temperature, UV-A/B	Not tested	Not tested

AD = *B. suis* was detected at all durations tested.
ND = *B. suis* was not detected at the shortest duration tested.
* *B. suis* was recovered during the 5-day test conducted 9/24/09 - 9/29/09 but not during the 5-day test conducted 8/23/09 - 8/28/09.
† *B. suis* was recovered during the 2-day test conducted 9/2/09 - 9/4/09 but not during the 2-day test conducted 9/18/09 - 9/20/09.

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