

# Evaluation of Sample Recovery Efficiency for Bacteriophage P22 on Fomites

Amanda B. Herzog,<sup>a,d</sup> Alok K. Pandey,<sup>a</sup> David Reyes-Gastelum,<sup>e</sup> Charles P. Gerba,<sup>d,f</sup> Joan B. Rose,<sup>c,d</sup> and Syed A. Hashsham<sup>a,b</sup>

Department of Civil and Environmental Engineering,<sup>a</sup> Center for Microbial Ecology,<sup>b</sup> Department of Fisheries and Wildlife,<sup>c</sup> Center for Advancing Microbial Risk Assessment,<sup>d</sup> and Center for Statistical Training and Consulting,<sup>e</sup> Michigan State University, East Lansing, Michigan, USA, and Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, Arizona, USA<sup>f</sup>

Fomites are known to play a role in the transmission of pathogens. Quantitative analysis of the parameters that affect sample recovery efficiency (SRE) at the limit of detection of viruses on fomites will aid in improving quantitative microbial risk assessment (QMRA) and infection control. The variability in SRE as a function of fomite type, fomite surface area, sampling time, application media, relative humidity (rH), and wetting agent was evaluated. To quantify the SRE, bacteriophage P22 was applied onto fomites at average surface densities of  $0.4 \pm 0.2$  and  $4 \pm 2$  PFU/cm<sup>2</sup>. Surface areas of 100 and 1,000 cm<sup>2</sup> of nonporous fomites found in indoor environments (acrylic, galvanized steel, and laminate) were evaluated with premoistened antistatic wipes. The parameters with the most effects on the SRE were sampling time, fomite surface area, wetting agent, and rH. At time zero (the initial application of bacteriophage P22), the SRE for the 1,000-cm<sup>2</sup> fomite surface area was, on average, 40% lower than that for the 100-cm<sup>2</sup> fomite surface area. For both fomite surface areas, the application medium Trypticase soy broth (TSB) and/or the laminate fomite predominantly resulted in a higher SRE. After the applied samples dried on the fomites (20 min), the average SRE was less than 3%. A TSB wetting agent applied on the fomite improved the SRE for all samples at 20 min. In addition, an rH greater than 28% generally resulted in a higher SRE than an rH less than 28%. The parameters impacting SRE at the limit of detection have the potential to enhance sampling strategies and data collection for QMRA models.

Nonporous fomites (inanimate or nonliving objects) can be important vehicles in the transmission of viral disease, especially for populated indoor environments, such as schools, day-care centers, nursing homes, hospitals, food preparation settings, or any civil infrastructure (4–6, 25, 32). Human exposure can occur through touching and transfer of pathogens present on the fomite to the hands and then to the mouth, nasopharynx, and eyes (5, 24). Exposure can also be from the inhalation of re-aerosolized organisms from contaminated fomites (5, 25). Controlling and remediating an indoor environment from an outbreak resulting from an accidental or intentional release of viruses can be challenging tasks (1, 25).

To declare an indoor environment “clean” after decontaminating it, quantification of the loss due to sample recovery that is specific to the method(s) used is essential for verifying the efficacy of the decontamination (16). Quantitative analyses of the parameters that affect sample recovery efficiency (SRE) from fomites are vital for implementing efficient sampling and detection methods (16). Infection transmission models that include the environmental dynamics (environmental conditions, human behavior, survival characteristics of the agent in the environment, etc.) can be used to make decisions on interventions for preventing viral outbreaks (19). Without a quantitative assessment of the abundance of such agents in the environment, generic intervention recommendations could be ineffective (4, 35).

Survival and SRE studies with viruses have generally been conducted on fomites at surface densities of 10<sup>2</sup> PFU/cm<sup>2</sup> or higher by applying virus stocks in volumes ranging from 5 to 500  $\mu$ l on fomite areas ranging from 0.38 to 32 cm<sup>2</sup> (Table 1). The use of higher initial titers is known to extend the virus survival rate on fomites (5). Under these optimal conditions, results may represent the upper limits of the SRE. The surface densities may also be lower than what has been studied so far and pose significant risk

(Table 1). However, the parameters affecting survival at very low surface densities are less well studied. To our knowledge, only two survival studies (3, 7) and two SRE studies (17, 36) have been conducted at surface densities ranging from 0.02 to 50 PFU or at the 50% tissue culture infective dose (TCID<sub>50</sub>)/cm<sup>2</sup> (Table 1; see also Table S1 in the supplemental material). These factors may have a significant effect on quantifying the risk to human health after decontamination.

The objective of this study was to evaluate the parameters that affect the SRE of bacteriophage P22, a surrogate for DNA viruses (20, 30), at concentrations close to the limit of detection. Bacteriophage P22 was chosen because it is a surrogate for DNA viruses such as adenovirus (13, 30), it meets many of the desired characteristics of a surrogate (20, 30, 33), and it has been used successfully by our group in environmental release and recovery studies (20, 30). We evaluated the variability of the SRE from the parameters, such as fomite type, fomite surface area, sampling time, application media, wetting agent, and relative humidity (rH). The results presented here have implications for sampling strategies and subsequent microbial risk assessment at low concentrations.

## MATERIALS AND METHODS

**Bacteriophage P22: preparation, application, and sample recovery.** Bacteriophage P22, which infects the bacterial host *Salmonella enterica*

Received 5 May 2012 Accepted 28 August 2012

Published ahead of print 31 August 2012

Address correspondence to Syed A. Hashsham, hashsham@egr.msu.edu.

Supplemental material for this article may be found at <http://aem.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AEM.01370-12

TABLE 1 Parameters from survival and SRE studies evaluating viruses applied to fomites<sup>a</sup>

Study type and organism	Sample concn	Fomite area (cm <sup>2</sup> )	Application vol (μl)	Surface density
<b>Survival studies</b>				
Alphaviruses	1.5 × 10 <sup>7</sup> –4.5 × 10 <sup>10</sup> PFU/ml	0.25	NR	6.4 × 10 <sup>6</sup> PFU/cm <sup>2</sup>
Ebola virus	1.5 × 10 <sup>7</sup> –4.5 × 10 <sup>10</sup> PFU/ml	0.25	NR	7.6 × 10 <sup>7</sup> PFU/cm <sup>2</sup>
Lassa virus	1.5 × 10 <sup>7</sup> –4.5 × 10 <sup>10</sup> PFU/ml	0.25	NR	5.6 × 10 <sup>7</sup> PFU/cm <sup>2</sup>
Astrovirus	2.0 × 10 <sup>6</sup> –2.5 × 10 <sup>7</sup> PFU/ml	1,3	20, 50	3.3 × 10 <sup>4</sup> –5 × 10 <sup>5</sup> PFU/cm <sup>2</sup>
Avian metapneumovirus	3.1 × 10 <sup>6</sup> –6.3 × 10 <sup>6</sup> TCID <sub>50</sub> /ml	1	10	3.4 × 10 <sup>4</sup> –6.3 × 10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Avian influenza virus	3.1 × 10 <sup>6</sup> –6.3 × 10 <sup>6</sup> TCID <sub>50</sub> /ml	1	10	3.4 × 10 <sup>4</sup> –6.3 × 10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Bacteriophage P22	1 × 10 <sup>9</sup> PFU/ml	10	10	10 <sup>7</sup> PFU/cm <sup>2</sup>
Calicivirus	10 <sup>7</sup> TCID <sub>50</sub> /ml	1	20	2.0 × 10 <sup>5</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Coronavirus	10 <sup>4</sup> –10 <sup>5</sup> MPN	1	10	10 <sup>4</sup> –10 <sup>5</sup> MPN/cm <sup>2</sup>
Coronavirus	10 <sup>7</sup> PFU/ml	0.79	10	1.3 × 10 <sup>5</sup> PFU/cm <sup>2</sup>
Feline calicivirus	10 <sup>9</sup> PFU/ml	25	NR	4 × 10 <sup>7</sup> PFU/cm <sup>2</sup>
Norwalk virus	10 <sup>6</sup> RT-PCRU/ml	25	NR	4 × 10 <sup>2</sup> RT-PCRU/cm <sup>2</sup>
Hepatitis A virus	10-fold dilution	0.79	10	N/A
Poliovirus	10-fold dilution	0.79	10	N/A
Hepatitis A virus	NR	1	20, 50, 100	N/A
Rotavirus	NR	1	20, 50, 100	N/A
Enteric adenovirus	NR	1	20, 50, 100	N/A
Poliovirus	NR	1	20, 50, 100	N/A
Rotavirus	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Poliovirus	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Bacteriophage f2	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Influenza A virus	2 × 10 <sup>8</sup> PFU/ml	NR	20	N/A
Influenza A virus	10 <sup>6</sup> TCID <sub>50</sub> /ml	1	10	10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Influenza A virus	1.5 × 10 <sup>8</sup> TCID <sub>50</sub> /ml	2	10	7.5 × 10 <sup>5</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Influenza A virus	10 <sup>3</sup> –10 <sup>4</sup> TCID <sub>50</sub> /0.1 ml	7.07–19.63	100	50–1.4 × 10 <sup>3</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Influenza B virus	10 <sup>3</sup> –10 <sup>4</sup> TCID <sub>50</sub> /0.1 ml	7.07–19.63	100	50–1.4 × 10 <sup>3</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Parainfluenza	1.5 × 10 <sup>9</sup> , 1.5 × 10 <sup>3</sup> , 1.5 × 10 <sup>4</sup> TCID <sub>50</sub> /ml	32	500	0.02–2.0 × 10 <sup>2</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Rhinovirus	10 <sup>7</sup> PFU/ml	0.79	10	1.3 × 10 <sup>5</sup> PFU/cm <sup>2</sup>
Zaire Ebola virus	1 × 10 <sup>6</sup> TCID <sub>50</sub> /ml	0.38	20	5.2 × 10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Lake Victoria Marburg virus	1 × 10 <sup>6</sup> TCID <sub>50</sub> /ml	0.38	20	5.2 × 10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
<b>SRE studies</b>				
Bacteriophage MS2	1 × 10 <sup>6</sup> PFU/ml	25	5	3.7 PFU/cm <sup>2</sup>
Feline calicivirus	7.0 × 10 <sup>5</sup> –1.3 × 10 <sup>6</sup> TCID <sub>50</sub> /100μl	25.8, 929, 5,290	20	26–10 <sup>4</sup> TCID <sub>50</sub> /cm <sup>2</sup>
Rotavirus	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Poliovirus	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Bacteriophage f2	10 <sup>3</sup> –10 <sup>5</sup> PFU/ml	250	Misted	N/A
Norovirus	2.0 × 10 <sup>7</sup> RT-PCRU/ml	10	100	2.0 × 10 <sup>3</sup> , 2.0 × 10 <sup>4</sup> RT-PCRU/cm <sup>2</sup>
Rotavirus	2.0 × 10 <sup>5</sup> RT-PCRU/ml	10	100	2.0 × 10 <sup>1</sup> , 2.0 × 10 <sup>2</sup> RT-PCRU/cm <sup>2</sup>
Rhinovirus	10 <sup>7</sup> PFU/ml	0.79	10	1.3 × 10 <sup>5</sup> PFU/cm <sup>2</sup>

(Continued on next page)

serovar Typhimurium LT2 (ATCC 19585), was provided by Charles P. Gerba (University of Arizona). Bacteriophage P22 is a double-stranded DNA (dsDNA) icosahedron-shaped virus with a short tail (52 to 60 nm in size) and belongs to the family *Podoviridae* (30). To prepare bacteriophage P22, 1 ml of bacteriophage P22 stock was added to 25 ml of the bacterial host, *S. Typhimurium*, at log phase in Trypticase soy broth (TSB) (Difco, Becton, Dickinson and Company, Sparks, MD). After a 24-h incubation at 37°C, 0.1 ml of lysozyme and 0.75 ml of EDTA were added to the solution and centrifuged at 2,390 × g for 10 min. The supernatant was filtered through a 0.45-μm filter (Millipore) to remove the bacterial cells and debris (30). Bacteriophage P22 was then diluted in suspensions of phosphate-buffered saline–Tween 80 (PBST) (Fisher Scientific, NJ), TSB, or sterile distilled water.

The fomites, simulating an indoor environment, included acrylic (Optix; Plaskolite Inc., Columbus, OH), galvanized steel (type 28 GA galvanized; MD Building Products, Oklahoma City, OK), and laminate (type 350, no. 60 matte finish; Wilsonart International Inc., Temple, TX) with

surface areas of 100 and 1,000 cm<sup>2</sup>. The fomites and testing area were disinfected with 70% ethanol, rinsed with sterile distilled water, and dried. Bacteriophage P22 was applied in PBST, TSB, or water on the fomite in a grid formation comprising 50 1-μl droplets. The average amount of bacteriophage P22 applied to the fomite was 433.1 ± 194.5 PFU, approximately 8.66 PFU/droplet, with average surface densities of 4.3 ± 1.9 PFU/cm<sup>2</sup> for the 100-cm<sup>2</sup> fomite and 0.4 ± 0.2 PFU/cm<sup>2</sup> for the 1,000-cm<sup>2</sup> fomite. The recovery materials, premoistened Fellowes screen cleaning wipes (no. 99703; Fellowes, Itasca, IL), are generally used to remove dirt, dust, and fingerprints from office equipment and are antistatic, nontoxic, and alcohol free. The premoistened wipes are made of crepe fabric (crepe material is treated as a trade secret by Fellowes) and wetted by the manufacturer with water and detergent (propylene glycol ethers). The premoistened wipes were cut into 48-cm<sup>2</sup> pieces using sterilized scissors and stored in sterile Whirl-Pak bags at room temperature during the experiment, lasting no more than 12 h. Fresh pieces were cut and used each day. The sampling was done by moving the premoistened wipes over the entire

TABLE 1 (Continued)

Application medium	Relative humidity (%)	Temp (°C)	Survival time or SRE	Reference
NR	30–40	20, 25	6–14 days	25
NR	30–40	20, 25	6–14 days	25
NR	30–40	20, 25	6–14 days	25
PBS or 20% FS	90 ± 5	4, 20	10–90 days	2
NR	NR	NR	1–6 days	38
NR	NR	NR	1–6 days	38
NR	50	25	36 days	20
NR	NR	NR	4–72 h	10
Cell culture medium	20 ± 3, 50 ± 3, 80 ± 3	4, 20, 40	0.25–28 days	8
PBS	55, 70	21	3–6 h	34
20% FS in PBS	75–88	22 ± 2	7 days	11
20% FS in PBS	75–88	22 ± 2	7 days	11
10% FS in saline	25 ± 5, 55 ± 5, 80 ± 5, 95 ± 5	5, 20, 35	4–96 h	21
10% FS in saline	25 ± 5, 55 ± 5, 80 ± 5, 95 ± 5	5, 20, 35	4–12 h	21
PBS or FS	50 ± 5, 85 ± 5, 90 ± 5	4, 20	30–60 days	1
PBS or FS	50 ± 5, 85 ± 5, 90 ± 5	4, 20	30–60 days	1
PBS or FS	50 ± 5, 85 ± 5, 90 ± 5	4, 20	5–60 days	1
PBS or FS	50 ± 5, 85 ± 5, 90 ± 5	4, 20	5–60 days	1
Distilled water, distilled water with 10% FS	NR	NR	0.75–1.5 h	18
Distilled water, distilled water with 10% FS	NR	NR	0.75–1.5 h	18
Distilled water, distilled water with 10% FS	NR	NR	0.75–1.5 h	18
NR	50–60	22 ± 2	6–24 h	22
Eagle minimal essential medium with 25 mM HEPES and Earle's salts	30–50	21–28	2 h–17 days	37
1% BSA	23–24	17–21	4–9 h	14
NR	35–40	27.8–28.3	24–48 h	3
NR	55–56	26.7–28.9	24–48 h	3
Minimum essential medium with Earle's salts	NR	22	6–10 h	7
Tryptose phosphate broth, bovine mucin, human nasal discharge	20 ± 5, 50 ± 5, 80 ± 5	20 ± 1	2–25 h	27
Guinea pig sera, tissue culture medium	55 ± 5	4, 22	14–50 days	23
Guinea pig sera, tissue culture medium	55 ± 5	4, 22	14–50 days	23
50% solution of TSB and dilution buffer (5 mM NaH <sub>2</sub> PO <sub>4</sub> and 10 mM NaCl)	45–60	20–22	7–40%	17
10% FS in PBS	NR	NR	3–71%	36
Distilled water, distilled water with 10% FS	NR	NR	16.8% ± 6%	18
Distilled water, distilled water with 10% FS	NR	NR	42.3% ± 1.9%	18
Distilled water, distilled water with 10% FS	NR	NR	10.6% ± 5.7%	18
10% PBS	NR	NR	10.3% ± 13.0%–51.9% ± 38.5%	28
10% PBS	NR	NR	5.4% ± 1.5%–57.7% ± 25.9%	28
Tryptose phosphate broth, bovine mucin, human nasal discharge	50 ± 5	22	40.3–98.4%	27

<sup>a</sup> NR, not reported; PBS, phosphate-buffered solution; RT-PCR, real-time PCR units; FS, fecal suspension; TCID<sub>50</sub>, median tissue culture infective dose; BSA, bovine serum albumin; MPN, most probable number; N/A, not applicable, not able to calculate surface density from information reported.

fomite twice (in perpendicular directions to each other). Two samples were taken, one immediately after the initial application (referred to as 0 min) and another after the samples were visibly dry (which was 20 min). The control experiments conducted with bacteriophage P22 suspensions to determine if the moistening agent had an effect on the viability of the virus indicated that, on average, 95% (range, 80 to 125%) of bacteriophage P22 could be recovered with inoculation directly onto the wipe and dissolution with PBST. Very high recovery rates were also seen at time zero on the fomites with no drying.

After sampling, the recovery material was placed into a 50-ml tube containing 5 ml of PBST and vortexed for 30 s. Bacteriophage P22 was assayed using a double-agar-layer method (39). The sample containing bacteriophage P22 (1 ml) was added to 2.5 ml of melted 1% agar overlay

(1 g Bacto agar/100 ml TSB) (Bacto agar; Difco, Becton, Dickinson and Company, Sparks, MD) with 0.3 ml of *S. Typhimurium* in the log phase. The solution was rolled by hand for mixing and immediately dispensed evenly onto 1.5% Trypticase soy agar (TSA) (Difco, Becton, Dickinson and Company, Sparks, MD) plates. After the overlay agar solidified, the plates were incubated at 37°C for 24 h; the number of PFU was then counted. A total of 324 plates were used in the conduction of the SRE experiments. These experiments included 3 fomite types, 2 sampling times, 3 application media, and 2 fomite surface areas. Each SRE measurement was made in triplicate and repeated on 3 different days. Because the numbers of PFU recovered were already very low, dilution of the samples was not necessary. For each sample recovery experiment, positive-control experiments were conducted in triplicate. Fifty 1- $\mu$ l droplets of bacterio-

phage P22 inoculated in 950  $\mu\text{l}$  of PBST (same as the extraction solution) were dispensed into a 1.5-ml microcentrifuge tube. The 1-ml bacteriophage P22 control was dispensed as described above.

**Single-agar-layer method for separating bacteriophage P22 survival from sample recovery.** When the survival of bacteriophage P22 on fomites was evaluated, 50 5- $\mu\text{l}$  droplets containing an estimated 3.96 PFU/droplet suspended in either TSB or water were applied on a polystyrene petri dish surface (100 by 15 mm) in a grid formation. An average of  $198 \pm 65$  PFU was applied to each plate, with an average surface density of  $2.5 \pm 0.9$  PFU/ $\text{cm}^2$ . For this experiment, the time of the first sampling (other than the initial sampling at time zero) was changed to 1 h instead of 20 min, because the 5- $\mu\text{l}$  droplets took longer to visibly dry on the petri dish. The samples were evaluated at 0, 1, 2, 4, 8, 12, and 24 h by implementing a single-agar-layer method. This method allowed us to evaluate the PFU remaining but eliminated the need to recover them from a surface, because bacteriophage P22 was directly applied on the petri dish surface. The assay consisted of dispensing 3 ml of melted 1% agar overlay (1 g Bacto agar/100 ml TSB) with 0.5 ml of *S. Typhimurium* in the log phase and 2 ml of TSB onto the petri dish surface where bacteriophage P22 was applied. After the overlay agar solidified, the plates were incubated at 37°C for 24 h, at which point the number of PFU was then counted. The experiment was conducted twice using 6 replicates per time point spanning 7 sampling time points and 2 application media (thus using a total of 168 plates). For each survival experiment, a positive-control experiment, which consisted of 50 5- $\mu\text{l}$  droplets of bacteriophage P22 inoculated in 750  $\mu\text{l}$  of PBST in 1.5-ml microcentrifuge tubes, was also included in triplicate. One milliliter of this positive control was plated as described above.

**Relative humidity and TSB wetting agent.** The rH and temperature in the laboratory were measured before each experiment with a digital rH and temperature meter (VWR Scientific Products). The average temperature of the laboratory during these experiments was  $20.8 \pm 0.23^\circ\text{C}$  (mean  $\pm$  standard deviation). The rH ranges of 9 to 23% and 28 to 32% were the natural rHs of the laboratory during the winter and summer months, respectively (see Fig. 3). For the rH range of 55 to 58%, a small laboratory space (14 ft by 7 ft by 9 ft) was equipped with a humidifier (Bonaire, Milford, MA).

Previous studies support the use of a wetting agent applied to the recovery material (wipe or swab) to enhance the SRE (9, 17, 18, 28). In a preliminary experiment, PBST and TSB were compared as wetting agents applied on the fomite surface to evaluate their effects on SRE enhancement at 20 min. There were no statistical differences between the SREs when PBST or TSB as the wetting agent was applied on the fomite ( $P = 0.232$ ,  $n = 27$ , Student's  $t$  test, data not shown). Hence, in further SRE experiments, a TSB wetting agent was used (this step is referred to as TSB wetting). Using a disposable spreader, 200 and 400  $\mu\text{l}$  of TSB was applied and uniformly distributed over 100- and 1,000- $\text{cm}^2$  fomite surface areas, respectively. The recovery material sampled both the disposable spreader and the fomite. The recovery materials were processed as described above. This experiment used a total of 162 plates consisting of 2 wetting conditions, 1 fomite surface area, 1 sampling time, 3 fomites, 3 application media, and 3 rHs. Each measurement was made in triplicate. A positive-control experiment was also conducted in triplicate as described previously for the SRE experiments.

**Percent surface recovery efficiency computations and statistical analysis.** The percent sample recovery efficiency was calculated as  $\%SRE = [N_{\text{assay}}(D)/N_{\text{control}}] \times 100$ , where %SRE is the sample recovery efficiency from the fomite,  $N_{\text{assay}}$  is the number of PFU counted on the agar plate from sampling the fomite,  $D$  is the dilution factor (the total extraction volume divided by the volume of sample assayed), and  $N_{\text{control}}$  is the number of units on the agar plate from the control experiment.

The data (%SRE) had considerable differences in variance, especially between 0 min and 20 min. Due to this, the data were transformed by adding 1 (to account for the zero values) and converted to a log scale. After analyzing the residuals, it was determined that the normality assumption of the residual did not fit the equation; therefore, the residuals were fitted

under the assumption of a gamma distribution. Two equations for the transformed outcome were used to study the relationships between fomite type, application medium, rH, and wetting condition.

$$\text{Log}(\%SRE + 1) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + e \quad (1)$$

For equation 1,  $\text{log}(\%SRE + 1)$  is the log-transformed SRE,  $X_1$  is an independent variable that denotes the fomite type so that  $X_1$  is a nominal variable with no numerical value (acrylic, laminate, and galvanized steel as categories) for which laminate was taken as the reference category in the analyses,  $X_2$  is an independent variable that denotes the application medium so that  $X_2$  is a nominal variable (PBST, TSB, and water as categories) for which water was taken as the reference category in the analyses,  $X_1 X_2$  is the interaction between fomite type and application medium, and  $e$  is the error term. The intercept  $\beta_0$  represents the average value of the log of the reference group, which in this case is the average value of the log of the reference categories laminate fomite and water medium. The terms  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are the regression coefficients known as the effects for the corresponding independent variables  $X_1$ ,  $X_2$ , and  $X_1 X_2$ , respectively.

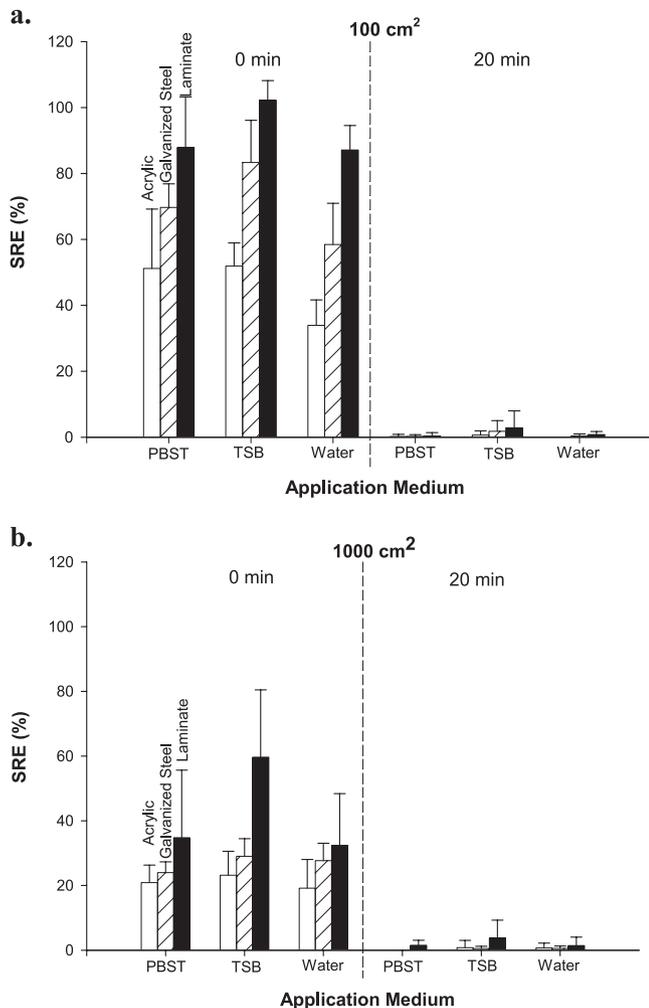
$$\text{Log}(\%SRE + 1) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_1 X_2 + \beta_6 X_2 X_3 + \beta_7 X_1 X_3 + e \quad (2)$$

In equation 2,  $\text{log}(\%SRE + 1)$  is the log-transformed SRE,  $X_1$  and  $X_2$  are as defined for equation 1,  $X_3$  is an independent variable that denotes the rH range so that  $X_3$  is a nominal variable (9 to 23%, 28 to 32%, and 55 to 58% as categories) for which 55 to 58% was taken as the reference category in the analyses,  $X_4$  is an independent variable that denotes the use of TSB wetting for the sample collection so that  $X_4$  is a nominal variable (no wetting and TSB wetting as categories) for which TSB wetting was taken as the reference category in the analyses, and  $e$  is the error term. As previously explained, the intercept  $\beta_0$  represents the average value of the log of the reference categories laminate fomite, water medium, 55 to 58% rH, and TSB wetting. The interaction terms are  $X_1 X_2$  (fomite type and application medium),  $X_2 X_3$  (application medium and rH), and  $X_1 X_3$  (fomite type and rH). The regression coefficients ( $\beta_{1-7}$ ) are known as the effects for the corresponding independent variables  $X_{1-4}$ ,  $X_1 X_2$ ,  $X_2 X_3$ , and  $X_1 X_3$ , respectively.

Because the independent variables used were nominal, dummy variables were used to compare the different categories to the corresponding reference categories. The dummy variable described the set of experimental conditions consisting of fomite type, application medium, rH, and wetting condition as a single entity, and we evaluated the SRE for each set to the next by treating two such sets as the reference (laminate and water). A regression was run using SAS 9.2 with the GLIMMIX procedure to evaluate the equations. The data were analyzed to evaluate the type III test of fixed effects (emanating from the factors being investigated) to determine the significance of each of the parameters specified in the model statement (26). Analyses of the model were performed on the wetting-condition, fomite surface area, and sampling-time groups. The patterns in the experimental data indicated differences to explore certain effects. This limited the error rates and avoided the cancellation of significant effects.

## RESULTS

**SREs of bacteriophage P22 from various fomites.** For the 100- $\text{cm}^2$  fomite surface area and the three application media (PBST, TSB, and water), the average SREs for the experimental data at 0 min were  $46\% \pm 6.9\%$  (SRE  $\pm$  standard deviation) for acrylic,  $70\% \pm 7.7\%$  for galvanized steel, and  $92\% \pm 6.4\%$  for laminate (Fig. 1a). The type III test of fixed effects (equation 1) for the 100- $\text{cm}^2$  fomite surface area at 0 min was significant for the fomite type ( $P < 0.0001$ ), the application medium ( $P < 0.0001$ ), and the interaction between fomite type and application medium ( $P = 0.0128$ ) (see Table S2 in the supplemental material). Based on equation 1, laminate yielded the highest SRE, while acrylic gave the lowest SRE regardless of which application medium was used.



**FIG 1** Experimental SREs of bacteriophage P22 from acrylic, galvanized steel, and laminate fomites. Bacteriophage P22 was applied in the media PBST, TSB, and water to fomite surface areas of 100 cm<sup>2</sup> (a) and 1,000 cm<sup>2</sup> (b). A premoistened wipe recovered bacteriophage P22 at the initial application time (0 min) and after drying (20 min). Each bar represents the average result from nine plates, and the error bars represent their standard deviations.

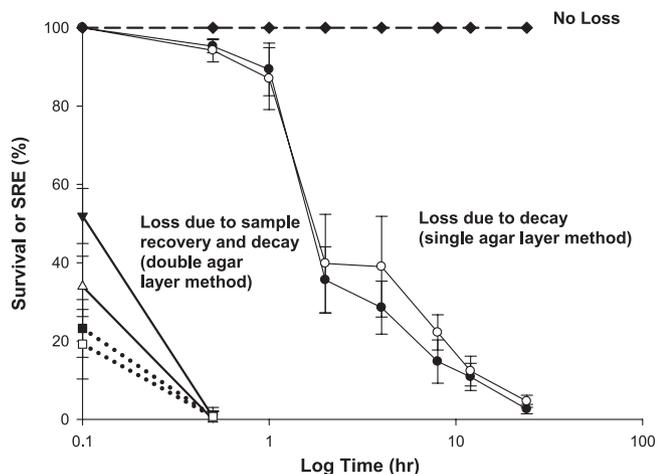
However, the use of TSB did result in a higher SRE than did the other media. PBST and TSB performed similarly on acrylic, while PBST and water performed similarly on laminate (see Table S3 in the supplemental material). At 20 min, the average SREs for acrylic, galvanized steel, and laminate were all less than 1%  $\pm$  0.9% for all application media (Fig. 1a). The type III test of fixed effects for the 100-cm<sup>2</sup> fomite surface area at 20 min was significant for the fomite type ( $P = 0.0047$ ) and application medium ( $P < 0.0001$ ) but not significant ( $P = 0.3589$ ) for the interaction between fomite type and application medium (see Table S4 in the supplemental material). For these conditions, the application medium significantly affected the SRE, and a higher SRE was observed for the application medium TSB. Similar to that at 0 min, the laminate resulted in a higher SRE, while acrylic resulted in a lower SRE. Similar results were observed on acrylic and galvanized steel when applied in the PBST medium (see Table S3).

Considering all application media for the 1,000-cm<sup>2</sup> fomite surface area, the average SREs for the experimental data at 0 min

were 21%  $\pm$  6.9% for acrylic, 26%  $\pm$  3.1% for galvanized steel, and 42%  $\pm$  19.2% for laminate (Fig. 1b). The type III test of fixed effects for the 1,000-cm<sup>2</sup> fomite surface area at 0 min was significant for the fomite type ( $P < 0.0001$ ), the application medium ( $P = 0.0037$ ), and the interaction between fomite type and application medium ( $P = 0.0998$ ) (see Table S5 in the supplemental material). The laminate fomite yielded the highest SRE, while the acrylic fomite gave the lowest SRE irrespective of the application medium. The use of TSB resulted in a higher SRE, while PBST and water had statistically equivalent SREs (see Table S6 in the supplemental material). At 20 min, the average SREs for the 1,000-cm<sup>2</sup> fomite surface area were 2%  $\pm$  1.4% or less for all surfaces and application media (Fig. 1b). The type III test of fixed effects for the 1,000-cm<sup>2</sup> fomite at 20 min was significant for the fomite type ( $P < 0.0001$ ) and application medium ( $P = 0.0053$ ) but not significant for the interaction between fomite type and application medium ( $P = 0.3720$ ) (see Table S7 in the supplemental material). The laminate fomite had the highest SRE, while acrylic and galvanized steel had lower and comparable SREs. The use of TSB and water as application media resulted in higher SREs than the use of PBST (see Table S6 in the supplemental material).

**SRE versus decay for bacteriophage P22.** The method we employed to determine the SREs included the loss due to decay. To separate this loss from the SREs, bacteriophage P22 was directly applied onto a petri dish (using TSB and water), and decay was quantified as described in Materials and Methods using the single-agar-layer method. The decay rates for bacteriophage P22 were  $7.97 \times 10^{-2} \text{ h}^{-1}$  when applied in TSB and  $6.81 \times 10^{-2} \text{ h}^{-1}$  when applied in water. After 1 h, when the 5- $\mu\text{l}$  droplets were visibly dry on the petri dish, the majority of the applied bacteriophage P22 was still infective (89.4%  $\pm$  6.7% in TSB and 87%  $\pm$  7.9% in water). These SREs were substantially higher than the SREs detectable at 20 min by employing the double-agar-layer method, which was 0% in water and 0.62%  $\pm$  1.3% in TSB for the 100-cm<sup>2</sup> acrylic fomite and 0.76%  $\pm$  1.6% in water and 0.69%  $\pm$  1.5% in TSB for the 1,000-cm<sup>2</sup> acrylic fomite. Even at 24 h, 2 to 5% of bacteriophage P22 was detectable using the single-agar-layer method (Fig. 2). These results indicate that a low or zero SRE may not always indicate an absence of the target, because SREs also include loss due to sample recovery.

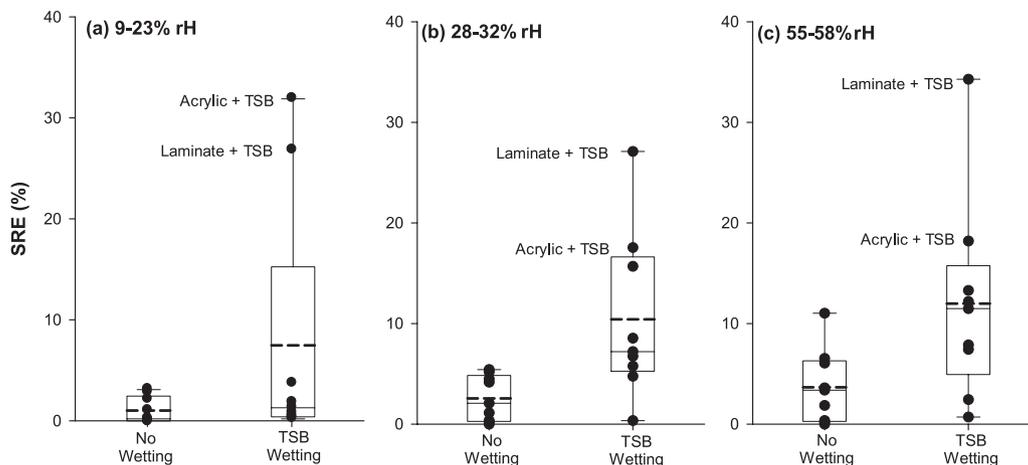
**Impact of wetting agent at varying relative humidity.** From the described experiment, it was clear that a significant portion of the bacteriophage P22 was still active on the fomite at 20 min and that the recovery material was unable to recover the dried sample. To enhance recovery, TSB was applied to the fomite as a wetting agent for SREs at 20 min (Fig. 3). Each point on the distribution represents the experimental data for each fomite type, application medium, rH, and TSB wetting combination. The type III test of fixed effects using equation 2 was significant for the application medium ( $P < 0.0001$ ), the rH ( $P < 0.0001$ ), the interaction between fomite type and application medium ( $P = 0.0001$ ), the interaction between fomite type and rH ( $P = 0.0048$ ), and the interaction between application medium and rH ( $P < 0.0001$ ). It was not significant for fomite type ( $P = 0.7634$ ). The results of using the TSB wetting step were significantly different from those when it was not used ( $P < 0.0001$ ) (see Table S8 in the supplemental material). The TSB wetting step improved the mean SRE for all cases. For both TSB wetting and no TSB wetting, bacteriophage P22 applied in TSB medium resulted in a higher SRE than when applied in the PBST and water. The exception to this was the



**FIG 2** Loss of bacteriophage P22 due to decay versus the loss due to sample recovery and decay. The survival rates of bacteriophage P22 are signified by circles (●, applied in TSB; ○, applied in water), and each point represents the mean and standard deviation of results from 12 plates. SREs from the 100-cm<sup>2</sup> fomite surface area are signified by triangles (▼, applied in TSB; △, applied in water), and the SREs from the 1,000-cm<sup>2</sup> fomite surface area are signified by squares (■, applied in TSB; □, applied in water). Each point of the SRE data represents the mean and standard deviation of results from nine plates.

acrylic and galvanized steel, where water gave a higher SRE at the 55 to 58% rH range (see Table S9 in the supplemental material).

Overall, regardless of the wetting agent, higher average SREs were primarily observed at rHs of 28 to 32% and 55 to 58%. The SRE values for both of these humidity ranges were not statistically different from each other. The mean predicted SRE was predominantly lower for the rH range of 9 to 23% than for those at the other two ranges. When water was used as the application medium, the highest average SRE was always obtained in the 55 to 58% rH range and the lowest in the 9 to 23% rH range (see Table S9 in the supplemental material). The effects of rH on SREs with the other application media (TSB and PBST) were less obvious than those with water.



**FIG 3** The experimental impact of rH and TSB wetting agents on the SREs of bacteriophage P22 after drying (20 min) on the 100-cm<sup>2</sup> fomite surface area. Bacteriophage P22 was sampled with premoistened wipes at rH ranges of 9 to 23% (a), 28 to 32% (b), and 55 to 58% (c). Each dot on the distribution represents the SRE from a single fomite (acrylic, galvanized steel, or laminate) and application medium (PBST, TSB, or water) combination. Those with the highest SREs are labeled. The solid lines in the box plots represent the median SREs, and the dashed lines represent the mean SREs. The box plot whiskers above and below the boxes indicate the 90th and 10th percentiles, respectively.

## DISCUSSION

Once decontamination has been conducted on an indoor site due to a viral outbreak or bioterrorism event, environmental monitoring and quantitative microbial risk assessment modeling will help determine the risk to human health and if the indoor site can be declared “clean” (15, 16). When monitoring fomites for viruses near the detection limit, the results from the linear regression equation suggest that the sampling priority should be for 100-cm<sup>2</sup> laminate fomite. At both sampling times (0 min and 20 min) and both fomite surface areas evaluated (100 cm<sup>2</sup> and 1,000 cm<sup>2</sup>), laminate resulted in higher SREs than those resulting from the other fomites under the same conditions. An increase in the fomite surface area from 100 to 1,000 cm<sup>2</sup> decreased the average SREs at 0 min by approximately 25% for acrylic, 40% for galvanized steel, and 50% for laminate (Fig. 1). A lower SRE for the larger surface area was expected, because the surface density was also lower. Previous studies have suggested that one method may not fit all scenarios, and in sampling for larger fomite surface areas the use of alternative recovery material may be more appropriate (12). Wipe methods are generally used for fomite surface areas of 10 to 25 cm<sup>2</sup>, but it is unknown what influence fomite surface area may have on the SRE (12). Low surface densities will require sampling of larger surface areas. Given that the SRE at the 1,000-cm<sup>2</sup> area was lower than the SRE at the 100-cm<sup>2</sup> area and SRE includes decay, sampling at low surface densities must be carried out with caution.

In general, the application medium TSB produced higher SREs than PBST and water. TSB is an organic medium used for the growth of bacteria and may have properties that are more stabilizing for the bacteriophage P22 on the fomite than on other media. It has been suggested that suspension in more complex media may affect resistance to desiccation (29). Most of the SRE studies reported earlier used organic media to suspend viral particles before application to the fomites (Table 1). The higher SREs in the TSB application medium suggest that the application medium may also influence the SRE, especially at low surface densities.

The most dramatic reduction in the average SRE of bacterio-

phage P22 from the fomite was with time (0 min versus 20 min). Initially, the inactivation of bacteriophage P22 could be the main reason for this loss in SRE when the sample was dry on the fomite (20 min). Most of the rapid inactivation occurs during the period of desiccation when bacteriophage P22 becomes less stable on the fomite than in a liquid medium (20). In addition, the concentration that was applied to the fomite was rather low and close to the limit of detection of the plaque assay. Virus survival rates increase with increases in concentration, which can stabilize the virus against environmental stressors (5). On average, less than 3% of bacteriophage P22 was recoverable after 20 min on the fomite. The SREs reported at 20 min varied widely from 3 to 98.4% (Table 1). Each of the studies had a different experimental approach for determining the SREs from fomites, which may account for the broad range of SREs reported. Keswick et al. (18), using cotton swabs, recovered rotavirus, poliovirus, and bacteriophage f2 immediately after applying the samples to the fomite. Similarly, cotton swabs were used to recover norovirus and rotavirus dried for 15 min on the fomite by Scherer et al. (28). Taku et al. (36) evaluated three methods, moistened cotton swabs or nylon filter, fomite contact with elution buffer and aspiration, and scraping with aspiration, to recover feline calicivirus dried for 15 min on the fomite. The recovery materials antistatic cloth, cotton swabs, and polyester swabs were evaluated by sampling bacteriophage MS2 dried for 45 min on the fomite (17). Sattar et al. (27) analyzed the SREs of human rhinovirus 14 dried on 1-cm-diameter disks for 1 h and then eluted the virus by submerging the disk in 1 ml of tryptose phosphate broth and sonicating. It is evident that the number of parameters influencing the SREs is rather large, posing a challenge for simple comparison.

A positive sample result indicates surface contamination and a potential risk of exposure. However, a negative result does not entirely ensure the absence of infectious agents and the absence of the potential risk of exposure (28). Following the same protocol, Masago et al. (20) found bacteriophage P22 to survive for 36 h on 10-cm<sup>2</sup> fomites (aluminum, ceramic, glass, plastic, stainless steel, and laminate) when applied at a surface density of approximately 10<sup>7</sup> PFU/cm<sup>2</sup> (Table 1). The decay rate of bacteriophage P22, reported by Masago et al. (20), for the plastic fomite was 5.2 × 10<sup>-3</sup> h<sup>-1</sup>. When eliminating the recovery method by applying the bacteriophage P22 (surface density, 2.5 ± 0.9 PFU/cm<sup>2</sup>) directly onto the petri dish (plastic fomite), 2 to 5% of bacteriophage P22 could be detected at 24 h. The decay rates for bacteriophage P22 on the petri dish were estimated to be 7.97 × 10<sup>-2</sup> h<sup>-1</sup> when applied in TSB and 6.81 × 10<sup>-2</sup> h<sup>-1</sup> when applied in water. The differences between the decay rates from Masago et al. (20) and this study were most likely due to the sample concentrations, since higher initial titers have been shown to extend survival on fomites (5). As seen in Fig. 2, at 1 h (5-μl droplets were visibly dry) an average of 88.2% ± 7.3% of the applied bacteriophage P22 was still active. The majority of the loss (40 to 60%) occurred between hours 1 and 2. Compared to this, the average SREs from the 100-cm<sup>2</sup> and 1,000-cm<sup>2</sup> acrylic surfaces at 20 min (1-μl droplets visibly dry) were less than 1% (Fig. 2). The survival of organisms on fomites is known to be agent specific and ranges from 0.75 h for rotavirus to 90 days for astrovirus (Table 1). Temperature, rH, fomite surface area, and sample concentration are all known to affect survival (5, 32, 40). Knowledge of the organisms' response to environmental stress on the fomite is important in determining the appropriate detection methods and employing clean-up strategies.

The results of the experiment designed to separate the decay from sample recovery (Fig. 2) revealed that for surface densities of 0.4 to 4 PFU/cm<sup>2</sup>, SREs were low due to poor efficiency of the recovery method rather than decay. The TSB wetting step improved the SREs for all cases at 20 min (see Table S9 in the supplemental material). The SRE results doubled in the majority of the cases, especially when the application medium was TSB. However, this TSB wetting step, the combination of the scraping from the disposable spreader and the application of the TSB wetting solution onto the fomite, may have physically dislodged the viral particles, resulting in a higher SRE than without the TSB wetting step (36). It can also be speculated that bacteriophage P22 may adhere strongly to the fomite surface after drying or may attach to an imperfection on the fomite so that the sampling material cannot desorb the virus off the fomite. Surface roughness has been shown to influence adhesion and cell retention to fomites, which can affect recovery (31, 38). In this study, surface roughness was not measured. The addition of the TSB wetting step demonstrates the potential to further desorb the viruses from the fomite and improve the SRE.

The rH and temperature are crucial parameters for virus survival on fomites (Table 1) (4, 5, 32). Higher SREs were observed for bacteriophage P22 at rH ranges of 28 to 32% and 55 to 58% regardless of the use of a wetting agent (Fig. 3). At an rH range of 9 to 23%, the lowest SREs were obtained (see Table S9 in the supplemental material). The combination of application medium and rH may also play a significant role in SRE. Bacteriophage P22 applied in water consistently had the highest SREs at 55 to 58% and the lowest SREs at 9 to 23%. However, for the rH ranges evaluated, its effect was not as obvious as for the other application media. The interaction between rH and application medium may be a useful parameter in implementing sampling strategies.

In summary, efficient sample recovery and detection methods are essential for determining the exposure of humans to viruses and the resulting risk in a contaminated indoor environment. The SREs of bacteriophage P22 from fomites at concentrations near the limit of detection were influenced most by the time of sampling, fomite surface area, the use of a wetting agent, and rH. The observations made here using bacteriophage P22 as a surrogate highlight some of the factors that must be considered when sampling for very low surface densities of threat agents. Understanding the contributions of decay and recovery in the overall measured SREs under various conditions and the parameters affecting them will assist in implementing appropriate sampling methods and decontamination strategies.

## ACKNOWLEDGMENTS

This study was supported by the Center for Advancing Microbial Risk Assessment funded by the U.S. Environmental Protection Agency and Department of Homeland Security grant R83236201.

We thank Rebecca Ives, Yoshifumi Masago, and Tomoyuki Shibata at Michigan State University for their technical support.

## REFERENCES

1. Abad FX, Pinto RM, Bosch A. 1994. Survival of enteric viruses on environmental fomites. *Appl. Environ. Microbiol.* 60:3704–3710.
2. Abad FX, et al. 2001. Potential role of fomites in the vehicular transmission of human astroviruses. *Appl. Environ. Microbiol.* 67:3904–3907.
3. Bean B, et al. 1982. Survival of influenza viruses on environmental surfaces. *J. Infect. Dis.* 146:47–51.
4. Boone SA, Gerba CP. 2010. The prevalence of human parainfluenza virus 1 on indoor office fomites. *Food Environ. Virol.* 2:41–46.

5. Boone SA, Gerba CP. 2007. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl. Environ. Microbiol.* 73:1687–1696.
6. Boxman ILA, et al. 2009. Environmental swabs as a tool in norovirus outbreak investigation, including outbreaks on cruise ships. *J. Food Prot.* 72:111–119.
7. Brady MT, Evans J, Cuartas J. 1990. Survival and disinfection of parainfluenza viruses on environmental surfaces. *Am. J. Infect. Control* 18:18–23.
8. Casanova LM, Jeon S, Rutala WA, Weber DJ, Sobsey MD. 2010. Effects of air temperature and relative humidity on coronavirus survival on surfaces. *Appl. Environ. Microbiol.* 76:2712–2717.
9. Centers for Disease Control and Prevention (CDC). 2002. Emergency preparedness and response: comprehensive procedures for collecting environmental samples for culturing *Bacillus anthracis*. <http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp>.
10. Clay S, Maherchandani S, Malik YS, Goyal SM. 2006. Survival on uncommon fomites of feline calicivirus, a surrogate of noroviruses. *Am. J. Infect. Control* 34:41–43.
11. D'Souza DH, et al. 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int. J. Food Microbiol.* 108:84–91.
12. Edmonds JM. 2009. Efficient methods for large-area surface sampling of sites contaminated with pathogenic microorganisms and other hazardous agents: current state, needs, and perspectives. *Appl. Microbiol. Biotechnol.* 84:811–816.
13. Enriquez C, et al. 2003. Bacteriophages MS2 and PRD1 in turfgrass by subsurface drip irrigation. *J. Environ. Eng.* 129:852–857.
14. Greatorex JS, et al. 2011. Survival of influenza A(H1N1) on materials found in households: implications for infection control. *PLoS One* 6:1–6. doi:10.1371/journal.pone.0027932.
15. Haas CN, Rose JB, Gerba CP. 1999. Quantitative microbial risk assessment. John Wiley, New York, NY.
16. Herzog AB, et al. 2009. Implications of limits of detection of various methods for *Bacillus anthracis* in computing risks to human health. *Appl. Environ. Microbiol.* 75:6331–6339.
17. Julian TR, Tamayo FJ, Leckie JO, Boehm AB. 2011. Comparison of surface sampling methods for virus recovery from fomites. *Appl. Environ. Microbiol.* 77:6918–6925.
18. Keswick BH, Pickering LK, Dupont HL, Woodward WE. 1983. Survival and detection of rotaviruses on environmental surfaces in day care centers. *Appl. Environ. Microbiol.* 46:813–816.
19. Li S, Eisenberg JNS, Spicknall IH, Koopman JS. 2009. Dynamics and control of infections transmitted from person to person through the environment. *Am. J. Epidemiol.* 170:257–265.
20. Masago Y, Shibata T, Rose JB. 2008. Bacteriophage P22 and *Staphylococcus aureus* attenuation on nonporous fomites as determined by plate assay and quantitative PCR. *Appl. Environ. Microbiol.* 74:5838–5840.
21. Mbithi JN, Springthorpe VS, Sattar SA. 1991. Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Appl. Environ. Microbiol.* 57:1394–1399.
22. Noyce JO, Michels H, Keevil CW. 2007. Inactivation of influenza A virus on copper versus stainless steel surfaces. *Appl. Environ. Microbiol.* 73:2748–2750.
23. Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. 2010. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. *J. Appl. Microbiol.* 109:1531–1539.
24. Rusin P, Maxwell S, Gerba C. 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J. Appl. Microbiol.* 93:585–592.
25. Sagripanti JL, Rom AM, Holland LE. 2010. Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. *Arch. Virol.* 155:2035–2039.
26. SAS Institute Inc. 2012. SAS/STAT(R) 9.22 user's guide. Type III test of fixed effects. [http://support.sas.com/documentation/cdl/en/statug/63347/HTML/default/viewer.htm#statug\\_glimmix\\_a0000001479.htm](http://support.sas.com/documentation/cdl/en/statug/63347/HTML/default/viewer.htm#statug_glimmix_a0000001479.htm).
27. Sattar SA, Karim YG, Springthorpe VS, Johnson-Lussenburg CM. 1987. Survival of human rhinovirus type 14 dried onto nonporous inanimate surfaces: effect of relative humidity and suspending medium. *Can. J. Microbiol.* 33:802–806.
28. Scherer K, et al. 2009. Application of a swab sampling method for the detection of norovirus and rotavirus on artificially contaminated food and environmental surfaces. *Food Environ. Virol.* 1:42–49.
29. Shams AM, Rose LJ, Hodges L, Arduino MJ. 2007. Survival of *Burkholderia pseudomallei* on environmental surfaces. *Appl. Environ. Microbiol.* 73:8001–8004.
30. Shen CP, et al. 2008. Evaluating bacteriophage P22 as a tracer in a complex surface water system: the Grand River, Michigan. *Environ. Sci. Technol.* 42:2426–2431.
31. Silva S, Teixeira P, Oliveira R, Azeredo J. 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. *J. Food Prot.* 71:1379–1385.
32. Sinclair R, Boone SA, Greenberg D, Keim P, Gerba CP. 2008. Persistence of category A select agents in the environment. *Appl. Environ. Microbiol.* 74:555–563.
33. Sinclair RG, Rose JB, Hashsham SA, Gerba CP, Haas CN. 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Appl. Environ. Microbiol.* 78:1969–1977.
34. Sizun J, Yu MWN, Talbot PJ. 2000. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. *J. Hosp. Infect.* 46:55–60.
35. Spicknall IH, et al. 2010. Informing optimal environmental influenza interventions: how the host, agent, and environment alter dominant routes of transmission. *PLoS Comput. Biol.* 6:11. doi:10.1371/journal.pcbi.1000969.
36. Taku A, et al. 2002. Concentration and detection of caliciviruses from food contact surfaces. *J. Food Prot.* 65:999–1004.
37. Thomas Y, et al. 2008. Survival of influenza virus on banknotes. *Appl. Environ. Microbiol.* 74:3002–3007.
38. Tiwari A, Patnayak DP, Chander Y, Parsad M, Goyal SM. 2006. Survival of two avian respiratory viruses on porous and nonporous surfaces. *Avian Dis.* 50:284–287.
39. US Environmental Protection Agency. 2001. Method 1602: male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. EPA 821-R-01-029. US Environmental Protection Agency, Washington, DC.
40. Vasickova P, Pavlik I, Verani M, Carducci A. 2010. Issues concerning survival of viruses on surfaces. *Food Environ. Virol.* 2:24–34.