

Higher concentrations of bacterial enveloped virus Phi6 can protect the virus from environmental decay

by

Ronald Bangiyev¹, Maxim Chudaev¹, Donald W. Schaffner² and Emanuel Goldman^{1*}

¹Department of Microbiology, Biochemistry & Molecular Genetics

New Jersey Medical School

Rutgers University

225 Warren Street

Newark, NJ 07103 USA

²Department of Food Science

Rutgers University

65 Dudley Road

New Brunswick, NJ 08901

*Corresponding author

tel: 973-972-4367

fax: 973-972-3644

email: egoldman@njms.rutgers.edu

21 ABSTRACT

22 Phage Phi6 is an enveloped virus considered as a possible non-pathogenic surrogate for SARS-
 23 CoV-2 and other viral pathogens in transmission studies. Higher input amounts of bacteriophage
 24 Phi6 are shown to delay and protect the phage from environmental decay, both when the phage are
 25 dried in plastic tubes, and when they are stored in saline solution at 4°C. When bacteriophage Phi6
 26 are placed in LB (Luria-Bertani) growth medium prior to placement on the plastic surface, viral
 27 recovery is not influenced by the starting concentration. The protection is reflected in longer half-
 28 lives of the phage at higher concentrations compared to lower. Because experiments supporting the
 29 possibility of fomite transmission of SARS-CoV-2 and other viruses rely upon survival of
 30 infectious virus following inoculation of various surfaces, high initial amounts of input virus on a
 31 surface may generate artificially inflated survival times compared to realistic lower levels of virus
 32 that a subject would normally encounter. This is not only because there are extra half-lives to go
 33 through at the higher concentrations, but also because the half-lives themselves are extended at the
 34 higher virus concentrations. It is important to design surface drying experiments for pathogens with
 35 realistic levels of input virus, and to consider the role of the carrier and matrix if the results are to be
 36 clinically relevant.

37

38 IMPORTANCE

39 During the COVID-19 pandemic, a lot of attention has been paid to the environmental decay of
 40 SARS-CoV-2 due to proposed transmission of the virus via fomites. However, published
 41 experiments have commenced with very high virus titer inoculums, an experimental design not
 42 representative of real-life conditions. The study described here evaluated the impact of initial virus
 43 titer on environmental decay of an enveloped virus, using a non-pathogenic surrogate for SARS-
 44 CoV-2, enveloped bacteriophage Phi6. We establish that higher concentrations of virus can protect
 45 the virus from environmental decay, depending on conditions. This has important implications for
 46 stability studies of SARS-CoV-2 and other viruses. Our results point to a limitation in the
 47 fundamental methodology that has been used to attribute fomite transmission for almost all
 48 respiratory viruses.

49 INTRODUCTION

50 Early in the COVID-19 pandemic, there was an intense focus on fomites (i.e., inanimate objects and
51 surfaces), as possible conduits for transmission of the causative agent, SARS-CoV-2. This was
52 because of a widely repeated contention that a person touching a freshly contaminated surface, not
53 washing hands, then quickly touching their mouth, nose or eyes, would lead to self-inoculation of
54 this respiratory virus. Consequently, considerable effort has been made to determine how long the
55 virus remains infectious after being deposited on various surfaces, and what conditions favor or
56 disfavor viability of the virus on these surfaces (Aboubakr et al, 2020; Biryukov et al., 2020; Bonil
57 et al., 2021; Chan et al, 2020; Chin et al, 2020; Kasloff et al., 2021; Kwon et al., 2021; Pastorino et
58 al., 2020; Paton et al., 2021; Riddell et al, 2020; van Doremalen et al, 2020).

59
60 In parallel to these studies, workers tested for the presence of viral RNA on surfaces in hospitals
61 treating COVID-19 patients (Chia et al., 2020; Guo et al., 2020; Ong et al., 2020; Piana et al., 2021;
62 Santarpia et al., 2020). These RT-PCR tests found viral RNA to be present on many surfaces (but
63 did not test for infectious virus, with one exception), and reinforced the perception that fomites
64 were indeed a significant risk factor for transmission of the disease.

65
66 In July 2020, one of us published (online) a Comment arguing that the risk of transmission of
67 SARS-CoV-2 by fomites was exaggerated (Goldman, 2020). New information that appeared since
68 then has strengthened this conclusion (Goldman, 2021). The basis for the argument was that the
69 amounts of virus used in experiments for determining how long infectious virus remains viable on
70 surfaces were orders of magnitude too large compared to what someone would actually encounter
71 in a real-world situation. Since the virus decays with a defined half-life depending on the surface,

72 the larger the inoculum, the more half-lives have to be gone through before there is less than one
73 infectious virus particle remaining on the surface. Smaller, more realistic inoculums would survive
74 through fewer half-lives, and therefore much less time would pass before the surface would be free
75 of infectious virus.

76
77 Among conditions favoring virus survival on surfaces was the observation that Bovine Serum
78 Albumin (BSA) protected the virus from environmental decay, and extended the time that the virus
79 remained viable (Pastorino et al., 2020). Similar observations with Bovine Serum Albumin were
80 also noted in experiments assessing viability of bacteriophage MS2, and enveloped bacteriophage
81 Phi6 in droplets (Lin et al., 2020). Bacteriophage Phi6 has been considered as a potential non-
82 pathogenic surrogate for enveloped viral pathogens like SARS-CoV-2 and Ebola virus (Fedorenko
83 et al., 2020; Whitworth et al., 2020).

84
85 The fact that Bovine Serum Albumin protected at least three viruses (SARS-CoV-2, phage MS2
86 and phage Phi6) from environmental decay made us wonder if higher concentrations of a virus
87 itself might similarly protect the virus from decay. Indeed, Marr and coworkers suggested the value
88 of "investigating the role of viral titer, which might affect aggregation and other characteristics, on
89 virus survival" (Lin et al., 2020).

90
91 There was already a suggestion that this might be the case for SARS-CoV-1. Table 1 in Lai et al
92 (2005) indicated that survival of SARS on paper, cotton gowns, and disposable gowns was much
93 greater for a 10^6 inoculum compared to 10^4 . At a 10^4 inoculum, infectious virus was not detectable
94 after 5 minutes, but with a 10^6 inoculum, infectious virus remained detectable for 24 hours. This

result suggested that the virus half-life was greatly extended with higher amounts of input virus.

In the work reported here, we have investigated the role of initial virus concentration on environmental decay of phage Phi6. We assayed survival of virus samples dried in plastic tubes for various lengths of time after drying. We show that higher input virus concentrations do indeed exhibit significantly larger percent survival and longer half-lives compared to lower virus input, however this effect is influenced by the inoculating matrix. The protective effect of higher virus concentrations also was observed for virus samples kept in solution at 4°C. This protective effect was not found for virus placed in Luria-Bertani (LB) growth medium (which contains Tryptone and Yeast Extract) before being placed in plastic tubes for drying.

MATERIALS AND METHODS

Phage preparation

Bacteriophage Phi6, and a bacterial host strain that it grows in, *Pseudomonas syringae* var phaseolicola HB10Y, were generous gifts of Lenny Mindich (now retired) of the Public Health Research Institute of Rutgers University. Cells grown overnight in LB medium in tubes shaken at 25°C were used in plaque assays with serial dilutions of virus to obtain countable numbers of plaques. Plaques assays were performed as described in Goldman (2015) except that the plates were incubated at room temperature (approximately 20°C). Luria-Bertani (LB) medium contained 10 g/l Tryptone, 5 g/l yeast extract, 10 g/l NaCl, and NaOH to adjust pH to 7.0 (<https://asm.org/getattachment/5d82aa34-b514-4d85-8af3-acabe6402874/LB-Luria-Agar-protocol-3031.pdf>).

118 Phage stocks were obtained by harvesting the top agar (6.5 g/l in LB) from one or two petri dishes
119 (containing 10 g/l agar in LB) exhibiting confluent lysis of the bacterial lawn. Saline solution (9 g/l
120 NaCl), 1 ml per plate, was added to the top agar, which was transferred to centrifuge tubes and
121 centrifuged at 20,000 x g for 5 minutes to remove agar and debris. This supernatant, stored at 4°C,
122 comprised the initial phage stock.

123

124 Preparation of phage stock in saline solution

125 An Amicon Ultra 100K filter device from Millipore was pre-rinsed with 4 ml of distilled water,
126 followed by subsequent washes with 70% ethanol and sterile saline solution, using centrifugation at
127 4°C in a fixed angle rotor at 5000 x g. Up to 4 ml of Phi6 initial phage stock was loaded on this
128 filter unit and centrifuged such that 200 µl volume remained above the filter (typical spin time 15-
129 20 min). Fluid below the filter was discarded, the filter unit was refilled with 3.8 ml of sterile saline
130 solution and the same centrifugation steps were repeated 5 times. The sample (saline stock) was
131 recovered in 200-400 µl volume and virus titer was determined by plaque assay.

132

133 Phage survival following drying in polypropylene tubes

134 An aliquot from the saline stock was subjected to a sequential series of 10-fold dilutions in saline.
135 Five µl of the stock, and 5 µl of each of the 10-fold dilutions, were placed near the bottom of 1.5 ml
136 capacity conical polypropylene Eppendorf microcentrifuge tubes (Corning). In our early
137 experiments, we allowed samples to air dry but switched to desiccation to save time. Samples were
138 desiccated under house vacuum (approximately 20 mm-Hg) and removed from the desiccator when
139 visually dry. Generally, this took between 15-20 minutes, with higher concentrations of phage
140 exhibiting shorter drying times, except for samples dried in LB medium where all samples took 19-

141 20 minutes to dry. We observed that there were no significant differences in patterns of phage
142 survival between air drying and desiccation. The data reported here were obtained from desiccated
143 samples.

144

145 Ambient room humidity was not controlled and varied in the building with a range between 10% to
146 45% over a period of six months, depending on the weather. However, for most of the experiments
147 reported here, humidity was around 15-25%. Humidity was monitored on a Holmes HHG-150
148 Comfort Check Hygrometer & Thermometer. Although humidity is known to significantly affect
149 environmental decay of Phi6 (Fedorenko et al, 2020; Lin et al, 2020; Whitworth et al, 2020), all
150 samples within a given experiment were subject to the same humidity, and our interest was only to
151 ascertain effects of initial viral concentration. Also, experiments measuring virus survival in real
152 world conditions, as has been done for SARS-CoV-2 (e.g., Mondelli et al, 2020; Ben-Shmuel et al,
153 2020), do not control for humidity, which is variable. Ambient room temperature was also not
154 controlled, but generally was maintained around 20°C.

155

156 Dried samples were reconstituted with 100 µl saline added to the Eppendorf tubes and vortexed.
157 The titer of viable virus remaining in each tube was then determined by plaque assay. Half-life for
158 the virus in a particular sample was calculated using the tool at:

159 <https://www.calculator.net/half-life-calculator.html?type=1&nt=25&n0=2300&t=60&t12=&x=45&y=11>

160

161 All experiments were repeated 1 – 3 times; representative experiments are shown in the tables.
162 Because of variations in conditions from experiment to experiment (such as humidity and initial
163 titer of phage stock), we did not pool results to obtain averages. However, the relative percent
164 survival in each experiment was generally consistent for the same time points, and the patterns were

165 reproducible within experimental limits.

166

167 For experiments with LB medium, the initial phage stock was subjected to a sequential series of 10-
168 fold dilutions in LB medium. The remainder of the protocol was the same as above.

169

170 Phage survival in solution

171 The serial dilutions used for the dry time experiments were stored at 4°C in the dark for later
172 testing. After the number of days as shown in the tables, the titer of the phage in each dilution was
173 determined by plaque assay, and compared to the initial titers as measured on day 1.

174

175 RESULTS AND DISCUSSION

176 Survival of Phi6 dried on plastic is increased at higher phage concentrations

177 Table 1 shows survival of Phi6 dried in plastic tubes and left for various lengths of times as a
178 function of initial virus concentration. The simple act of drying the phage led to loss of nearly all
179 viable phage at the lower phage input concentrations (lines 3 and 4), while having no significant
180 effect (93% recovery) on the highest phage concentration tested (line 1), and a small effect (70%
181 recovery) on a 10-fold lower initial phage concentration (line 2).

182

183 Similar patterns of protection by higher initial phage concentrations were also seen for all
184 subsequent lengths of time the phage remained dry in the tube. At 15 minutes dry time, we began to
185 see some loss of survival from the most concentrated initial virus input (line 5, 83% recovery)
186 compared to a 10-fold lower virus input (line 6, 27% recovery), As was seen for the samples dried
187 and assayed immediately, the lowest virus inputs led to loss of almost all viable phage (line7, 11%

188 recovery, line 8, none recovered).

189

190 After 30 or 60 minutes of dry-time, the highest initial phage inputs began to show significant
191 environmental decay, with just 43% recovery after 30 minutes (line 9), and 38% recovery at 60
192 minutes (line 13). But even more substantial environmental decay was observed for the lower input
193 virus samples (Lines 10-12 for 30-minute dry time, lines 14-16 for 60 minutes).

194

195 For those samples with measurable virus survival, we were able to calculate the half-lives of virus
196 in those samples (Table 1), which were commensurate to the percent survival observed. That is, at
197 the higher phage initial input levels, half-lives were much longer than the half-lives at lower initial
198 phage input, e.g., compare line 5 (57 minute half-life) to line 6 (8 minute half-life), or line 9 (24
199 minute half-life) to line 11 (6 minute half-life), or line 13 (42 minute half-life) to line 14 (13 minute
200 half-life).

201

202 The results in Table 1 show that the higher phage input concentrations delay and protect dried
203 phage from environmental decay compared to lower phage input concentrations. This effect is also
204 visualized in Figure 1A. The black circles show the relationship between the log PFU (plaque
205 forming units) inoculated onto the surface versus the level recovered. The dashed line represents the
206 best fit regression line. The solid line represents a relationship where all inoculated viruses would
207 be recovered (i.e., 100% recovery). It's clear from the difference between the slopes of the two lines
208 that recovery is progressively lower as the inoculation level declines. In those experiments where
209 inoculated virus was not recovered, no recovery (0 PFU) is visualized in the figure at $-1 \log$ PFU.

210

211 Survival of Phi6 in saline at 4°C is increased at higher phage concentrations

212 We decided to test whether there was an effect of phage concentration on virus stability in solution,
 213 as was seen for phage dried on surfaces. Table 2 shows little decay of phage in solution at 4°C at
 214 the highest concentrations tested after 20 days (line 1), or about half decay after 56 days (line 4).
 215 But as the input concentration is reduced, decay increases at both time points (lines 2 and 5), with
 216 percent recovery in single digits for the lowest input phage samples (lines 3 and 6). These results
 217 can also be visualized in Figure 1B. As in Figure 1A, the black circles show the relationship
 218 between the log PFU inoculated onto the surface versus the level recovered. The dashed line
 219 represents the best fit regression line, and the solid line represents a relationship where all
 220 inoculated viruses would be recovered (i.e., 100% recovery). As in Figure 1A, it's clear from the
 221 difference between the slopes of the two lines that recovery is progressively lower as the
 222 inoculation level declines. These experiments had no trials where inoculated virus was not
 223 recovered.

225 Protection of Phi6 from decay by LB medium

226 In our earliest experiments, we used the "initial phage stock" (see Materials and Methods), which
 227 did show protection from decay at the higher phage concentrations. But we realized that this stock
 228 also had some level of LB medium present, derived from the soft top agar layer containing the
 229 lysate in phage preparation. Therefore, dilutions of the initial phage stock also diluted part of the
 230 medium the phage had been grown in, which is what prompted us to develop the filtration method
 231 described in the Materials and Methods. This allowed the phage to be tested without residual
 232 components of the growth medium, which could affect phage survival.

233

234 Nevertheless, we wondered whether growth medium itself might also protect the phage from decay,
 235 similar to observations with BSA (Lin et al., 2020). Table 3 shows that this is indeed the case.
 236 When phage samples were diluted in LB medium instead of saline, phage were essentially
 237 completely protected from decay following a half hour dry time (lines 1-5). This is in marked
 238 contrast to the results when phage were diluted in saline (Table 1). After extending the dry time for
 239 LB-containing samples to 24 hours, environmental decay was now evident in the samples, with
 240 recoveries of phage ranging between 16-39% (lines 6-10). There was no effect of varying the
 241 amount of initial input phage, showing that LB medium delays and protects even lower
 242 concentrations of phage from environmental decay. This is evident from Figure 1C, which shows
 243 that as the level of log PFU inoculated declines, the log PFU declines proportionally, and the slope
 244 of the regression line (dashed line) is essentially parallel to the line of 100% recovery (solid black
 245 line). The regression lies slightly under the line of 100% recovery, which indicates that most, but
 246 not all the virus inoculated was recovered with the same rate of environmental decay, but this varied
 247 slightly from experiment to experiment. These experiments also had no trials where inoculated
 248 virus was not recovered.
 249
 250 Our data demonstrate that bacteriophage Phi6, an enveloped virus that has been considered a
 251 potential non-pathogenic surrogate for SARS-CoV-2 transmission studies, exhibits a slower loss of
 252 infectivity from environmental decay by higher initial virus concentrations, depending upon the
 253 carrier. This slower loss of infectivity is true both when the phage are dried on plastic surfaces, and
 254 when the phage are left in saline solution in the refrigerator. The protection at higher phage
 255 concentrations is reflected in longer half-lives compared to lower phage concentrations.
 256

257 Phage survival on dried surfaces can be greatly affected by the type of surface, by temperature and
 258 humidity, and by the medium containing the phage (Lin et al., 2020; Fedorenko et al., 2020;
 259 Whitworth et al., 2020). Indeed, we observed dramatic delay and protection of phage by LB growth
 260 medium, which superseded the effects of initial phage concentration. Thus, an important limitation
 261 of our results is that we do not know what effect (if any) on phage survival would result from other
 262 natural additions, such as mucous for example, which has been tested for SARS-CoV-2 (Matson et
 263 al., 2020).

264
 265 A recent study compared environmental stability of dried SARS-CoV-2 for two different initial
 266 virus inocula (4×10^5 versus 4×10^3) on stainless steel, and did not observe additional protection in
 267 the rate of decay at the higher concentration (Paton et al., 2021). The SARS-CoV-2 samples used in
 268 this study were in growth medium including fetal bovine serum, which may be more comparable to
 269 our results for Phi6 in LB medium. Also, their lower tested concentration (4×10^3) may still have
 270 been too high to observe accelerated decay at lower virus concentrations.

271
 272 Our results have implications for experiments measuring SARS CoV-2 survival on surfaces as
 273 purported sources of transmission. High initial amounts of input virus on a surface may generate
 274 artificially inflated survival times compared to realistic lower levels of virus that a subject would
 275 normally encounter, not only because there are extra half-lives to go through at the higher
 276 concentrations, but also because the half-lives themselves are extended at the higher virus
 277 concentrations.

278
 279 The implications of our findings are also relevant for experiments supporting transmission of

280 respiratory viruses by fomites in general. With the exception of Respiratory Syncytial virus, the
 281 belief that most, if not all, of these viruses can be spread by fomites is based solely on dried virus
 282 stability experiments (e.g. Kutter et al, 2018). In the case of rhinovirus, the major cause of the
 283 common cold, an early report demonstrating experimental fomite transmission used unrealistic
 284 conditions (Gwaltney and Hendley, 1982). A subsequent study closer to real-life conditions
 285 disproved this route of transmission, at least to a first approximation (Dick et al, 1987). Apparent
 286 low efficiency of virus transfer by fingers also needs to be considered when assessing the
 287 possibility of fomite transmission, as contact with hands might inactivate some viruses (Weber and
 288 Stilianakis, 2021).

289

290 We do not know why higher concentrations of input Phi6 protect the virus from decay, or why it is
 291 influenced by the carrier. It could just be a result of higher protein levels in the phage solution
 292 buffering the phage particles from the effects of drying out. Marr and coworkers pointed out that as
 293 liquid evaporates, the concentration of solutes (in our case, salt) increases in the microenvironment
 294 (Lin et al., 2020), which may affect survival. Further, virus aggregation at higher concentrations is
 295 likely, and such aggregates may be protective (Gerba and Betancourt, 2017), or simply manifest as
 296 protective because clusters of viruses will be counted as single PFU, but behave kinetically as more
 297 resistant viruses. Whatever the cause for this protection, it is even more imperative to design
 298 surface drying experiments for pathogens with realistic levels of input virus, and account for the
 299 effects of carrier and matrix if the results are to be clinically relevant.

300

301 ACKNOWLEDGEMENTS

302 Funding sources for EG's laboratory were grants from the New Jersey Alliance for Clinical and

303 Translational Science (NJ ACTS), and the Rutgers Research Council. We are grateful to Matthew
304 Igo for advice and sharing a lab protocol for Phi6 plaque assays. We acknowledge with gratitude
305 the many helpful comments and insights offered by Wlodek Mandecki.

306

307 AUTHOR CONTRIBUTIONS

308 RB performed the dry time experiments; EG performed the solution experiments with assistance
309 from RB. MC assisted in preparation of materials used and developed the filtration method for
310 generating saline solutions of phage from LB stocks. First draft of the manuscript was prepared by
311 EG, with input from a report by RB. DS generated the figure, and assisted in interpretation of the
312 experiments and revisions of the manuscript. All authors reviewed the manuscript, made
313 corrections, and approved the final version. Overall supervision of the project was under the
314 direction of EG.

315

316 REFERENCES

317 Aboubakr, HA, Sharafeldin, TA, Goyal, SM. Stability of SARS-CoV-2 and other coronaviruses in
318 the environment and on common touch surfaces and the influence of climatic conditions: A review.
319 Transbound Emerg Dis. 2020; 00: 1– 17. <https://doi.org/10.1111/tbed.13707>

320

321 Ben-Shmuel A, Brosh-Nissimov T, Glinert I, et al. Detection and infectivity potential of severe
322 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) environmental contamination in isolation
323 units and quarantine facilities. Clin Microbiol Infect. 2020; 26:1658-1662.

324 <https://doi.org/10.1016/j.cmi.2020.09.004>

325

326 Biryukov, J., Boydston, J. A., Dunning, R. A., Yeager, J. J., Wood, S., Reese, A. L., Ferris, A.,
327 Miller, D., Weaver, W., Zeitouni, N. E., Phillips, A., Freeburger, D., Hooper, I., Ratnesar-Shumate,
328 S., Yolitz, J., Krause, M., Williams, G., Dawson, D. G., Herzog, A., Dabisch, P., ... Altamura, L.
329 A. (2020). Increasing Temperature and Relative Humidity Accelerates Inactivation of SARS-CoV-2
330 on Surfaces. mSphere, 5(4), e00441-20. <https://doi.org/10.1128/mSphere.00441-20>
331
332 Bonil, L.; Lingas, G.; Coupeau, D.; Lucet, J.-C.; Guedj, J.; Visseaux, B.; Muylkens, B. Survival of
333 SARS-CoV-2 on Non-Porous Materials in an Experimental Setting Representative of Fomites.
334 Coatings 2021, 11, 371. <https://doi.org/10.3390/coatings11040371>
335
336 Chan KH, Sridhar S, Zhang RR, et al. (2020) Factors affecting stability and infectivity of SARS-
337 CoV-2. J Hosp Infect;106(2):226-231. <https://doi.org/10.1016/j.jhin.2020.07.009>
338
339 Chia PY, Coleman KK, Tan YK, et al. Detection of air and surface contamination by SARS-CoV-2
340 in hospital rooms of infected patients. Nat Commun. 2020;11(1):2800. Published 2020 May 29.
341 <https://doi.org/10.1038/s41467-020-16670-2>
342
343 Chin AWH, Chu JTS, Perera MRA, et al. Stability of SARS-CoV-2 in different environmental
344 conditions. Lancet Microbe. 2020;1(1):e10. [https://doi.org/10.1016/S2666-5247\(20\)30003-3](https://doi.org/10.1016/S2666-5247(20)30003-3)
345
346 Dick EC, Jennings LC, Mink KA, Wartgow CD, Inhorn SL. Aerosol transmission of rhinovirus
347 colds. J Infect Dis. 1987; 156: 442-8. <https://doi.org/10.1093/infdis/156.3.442>
348

349 Fedorenko, A., Grinberg, M., Orevi, T. et al. Survival of the enveloped bacteriophage Phi6 (a
350 surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Sci Rep*
351 10, 22419 (2020). <https://doi.org/10.1038/s41598-020-79625-z>
352

353 Gerba CP, Betancourt WQ. Viral Aggregation: Impact on Virus Behavior in the Environment.
354 *Environ Sci Technol*. 2017 Jul 5;51(13):7318-7325. <https://doi.org/10.1021/acs.est.6b05835>
355

356 Goldman E (2015) Plaque Assay for Bacteriophage. In *Practical Handbook of Microbiology*, 3rd
357 Edition (Goldman E and Green L, eds.), CRC Press, Boca Raton, FL, pp. 93-97.
358

359 Goldman E. Exaggerated risk of transmission of COVID-19 by fomites. *Lancet Infect Dis*. 2020;
360 20:892-893. [https://doi.org/10.1016/S1473-3099\(20\)30561-2](https://doi.org/10.1016/S1473-3099(20)30561-2)
361

362 Goldman E. SARS Wars: the fomites strike back. *Appl Environ Microbiol*. 2021 Apr
363 30:AEM.00653-21. <https://doi.org/10.1128/AEM.00653-21>.
364

365 Guo Z, Wang Z, Zhang S, et al. Aerosol and Surface Distribution of Severe Acute Respiratory
366 Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020. *Emerging Infectious Diseases*.
367 2020;26(7):1583-1591. <https://doi.org/10.3201/eid2607.200885>.
368

369 Gwaltney JM Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated
370 surfaces. *Am J Epidemiol*. 1982 Nov;116(5):828-33.
371 <https://doi.org/10.1093/oxfordjournals.aje.a113473>

372

373 Kasloff SB, Leung A, Strong JE, Funk D, Cutts T. Stability of SARS-CoV-2 on critical personal
374 protective equipment. Sci Rep. 2021 Jan 13;11(1):984. [https://doi.org/10.1038/s41598-020-](https://doi.org/10.1038/s41598-020-80098-3)
375 [80098-3](https://doi.org/10.1038/s41598-020-80098-3).

376

377 Kutter JS, Spronken MI, Fraaij PL, Fouchier RA, Herfst S. Transmission routes of respiratory
378 viruses among humans. Curr Opin Virol. 2018; 28:142-151.
379 <https://www.sciencedirect.com/science/article/pii/S1879625717301773?via%3Dihub>

380

381 Kwon T, Gaudreault NN, Richt JA. Environmental Stability of SARS-CoV-2 on Different Types of
382 Surfaces under Indoor and Seasonal Climate Conditions. Pathogens. 2021 Feb 18;10(2):227.
383 <https://doi.org/10.3390/pathogens10020227>

384

385 Lai MY, Cheng PK, Lim WW. Survival of severe acute respiratory syndrome coronavirus. Clin
386 Infect Dis. 2005; 41: e67-71. <https://doi.org/10.1086/433186>

387

388 Lin K, Schulte CR, Marr LC (2020) Survival of MS2 and Φ 6 viruses in droplets as a function of
389 relative humidity, pH, and salt, protein, and surfactant concentrations. PLoS ONE 15(12):
390 e0243505. <https://doi.org/10.1371/journal.pone.0243505>

391

392 Matson MJ, Yinda CK, Seifert SN et al. Effect of Environmental Conditions on SARS-CoV-2
393 Stability in Human Nasal Mucus and Sputum. Emerg Infect Dis. 2020; 26: 2276-8. 183
394 <https://dx.doi.org/10.3201/eid2609.202267>

395

396 Mondelli MU, Colaneri M, Seminari EM, et al. Low risk of SARS-CoV-2 transmission by fomites
397 in real-life conditions Lancet Infect Dis. 2020; (published online September 29.)
398 [https://doi.org/10.1016/S1473-3099\(20\)30678-2](https://doi.org/10.1016/S1473-3099(20)30678-2)
399

400 Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, Marimuthu K. Air, Surface
401 Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory
402 Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. JAMA. 2020 Apr
403 28;323(16):1610-1612. <https://doi.org/10.1001/jama.2020.3227>.
404

405 Pastorino B, Touret F, Gilles M et al. Prolonged Infectivity of SARS-CoV-2 in Fomites. Emerg
406 Infect Dis. 2020; 26: 2256-7. <https://dx.doi.org/10.3201/eid2609.201788>
407

408 Paton S, Spencer A, Garratt I, Thompson K-A, Dinesh I, Aranega-Bou P, Stevenson D, Clark S,
409 Dunning J, Bennett A, Pottage T. Persistence of SARS-CoV-2 virus and viral RNA in relation to
410 surface type and contamination concentration. Appl Environ Microbiol. May 2021, AEM.00526-21;
411 <https://aem.asm.org/content/aem/early/2021/05/03/AEM.00526-21.full.pdf>
412

413 Piana, A., Colucci, M. E., Valeriani, F., Marcolongo, A., Sotgiu, G., Pasquarella, C., Margarucci, L.
414 M., Petrucca, A., Gianfranceschi, G., Babudieri, S., Vitali, P., D'Ermo, G., Bizzarro, A., De Maio,
415 F., Vitali, M., Azara, A., Romano, F., Simmaco, M., & Romano Spica, V. (2021). Monitoring
416 COVID-19 Transmission Risks by Quantitative Real-Time PCR Tracing of Droplets in Hospital
417 and Living Environments. mSphere, 6(1), e01070-20. <https://doi.org/10.1128/mSphere.01070-20>

418

419 Riddell S, Goldie S, Hill A, et al. The effect of temperature on persistence of SARS-CoV-2 on
420 common surfaces. Virol J 2020; 17: 145. <https://doi.org/10.1186/s12985-020-01418-7>

421

422 Santarpia, J.L., Rivera, D.N., Herrera, V.L. et al. Aerosol and surface contamination of SARS-CoV-
423 2 observed in quarantine and isolation care. Sci Rep 10, 12732 (2020).
424 <https://doi.org/10.1038/s41598-020-69286-3>

425

426 van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A,
427 Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. (2020) Aerosol and
428 Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 382:1564-1567.
429 <https://www.nejm.org/doi/full/10.1056/nejmc2004973>

430

431 Weber TP, Stilianakis NI. Fomites, hands, and the transmission of respiratory viruses. J Occup
432 Environ Hyg. 2021; 18:1-3. <https://doi.org/10.1080/15459624.2020.1845343>

433

434 Whitworth C, Mu Y, Houston H, Martinez-Smith M, Noble-Wang J, Coulliette-Salmond A, Rose
435 L. 2020. Persistence of bacteriophage phi 6 on porous and nonporous surfaces and the potential for
436 its use as an Ebola virus or coronavirus surrogate. Appl Environ Microbiol 86:e01482-20.
437 <https://doi.org/10.1128/AEM.01482-20>.

438

439

440

TABLE 1

Extent of survival of Phi6 dried on plastic is increased at higher phage concentrations

Line	Time dry (min)	Inoculum	Recovered	% Recovered	Half-life (min)
1	0	6×10^3	5.6×10^3	93	
2	0	6×10^2	4.2×10^2	70	
3	0	60	1	2	
4	0	6	0	0	
5	15	1.2×10^4	1×10^4	83	57
6	15	1.2×10^3	3.2×10^2	27	8
7	15	1.2×10^2	13	11	5
8	15	12	0	0	
9	30	4.7×10^4	2×10^4	43	24
10	30	4.7×10^3	7.6×10^2	16	11
11	30	4.7×10^2	18	4	6
12	30	47	0	0	
13	60	3.2×10^4	1.2×10^4	38	42
14	60	3.2×10^3	1.4×10^2	4	13
15	60	3.2×10^2	0	0	
16	60	32	0	0	

Amounts of phage Phi6 as shown in the column "Inoculum" were dried in polypropylene tubes.

Samples were reconstituted in 100 μ l saline at the times after drying shown in the table. The

amounts of viable phage remaining in the reconstituted samples were determined and shown in the

column "Recovered". Half-life was calculated as described in Materials and Methods.

449 TABLE 2

450 Survival of Phi6 in saline at 4°C is increased at higher phage concentrations

<u>Line</u>	<u>Time (days)</u>	<u>Initial input</u>	<u>Recovered</u>	<u>% Recovered</u>	<u>Half-life (days)</u>
1	20	2×10^4	1.9×10^4	95	270
2	20	2×10^3	2.9×10^2	15	7
3	20	2×10^2	14	7	5
4	56	1.2×10^4	5.4×10^3	45	49
5	56	1.2×10^3	1.4×10^2	12	18
6	56	1.2×10^2	2	2	9

451

452 Amounts of phage Phi6 in saline shown in the column "Initial input" were placed in tubes on day 1

453 and kept at 4°C for the times indicated, at which point the amounts of viable phage remaining in

454 the tubes were determined and shown in the column "Recovered". Half-life was calculated as

455 described in Materials and Methods.

456

457

458 TABLE 3

459 Protection of Phi6 from decay by LB medium dried on plastic

Line	Time dry (hours)	Inoculum	Recovered	% Recovered	Half-life (hours)
1	0.5	1.8×10^5	1.5×10^5	83	
2	0.5	1.8×10^4	1.5×10^4	83	
3	0.5	1.8×10^3	2.2×10^3	122	
4	0.5	1.8×10^2	1.5×10^2	83	
5	0.5	18	18	100	
6	24	9×10^4	2×10^4	22	11
7	24	9×10^3	3.5×10^3	39	18
8	24	9×10^2	2.5×10^2	28	13
9	24	90	14	16	9
10	24	9	2	22	11

460

461 Samples were generated as in Table 1, except that the serial dilutions of the phage stock were in LB

462 medium instead of saline. Half-life was calculated as described in Materials and Methods.

463

464 **Legend to Figure 1**

465 Relationship between the log PFU inoculated versus the level recovered (black circles) for (A)

466 bacteriophage phi 6 on plastic in saline at room temperature, (B) bacteriophage phi 6 in saline at

467 4°C and (C) bacteriophage phi 6 on plastic in Luria-Bertani broth at room temperature. The dashed

468 lines represent the best fit regression line for each dataset. The solid line represents the line of

469 perfect recovery where all inoculated viruses would be recovered (i.e., 100% recovery). Samples in

470 those experiments where inoculated virus was not recovered (0 PFU) are visualized in the figure at

471 -1 log PFU.

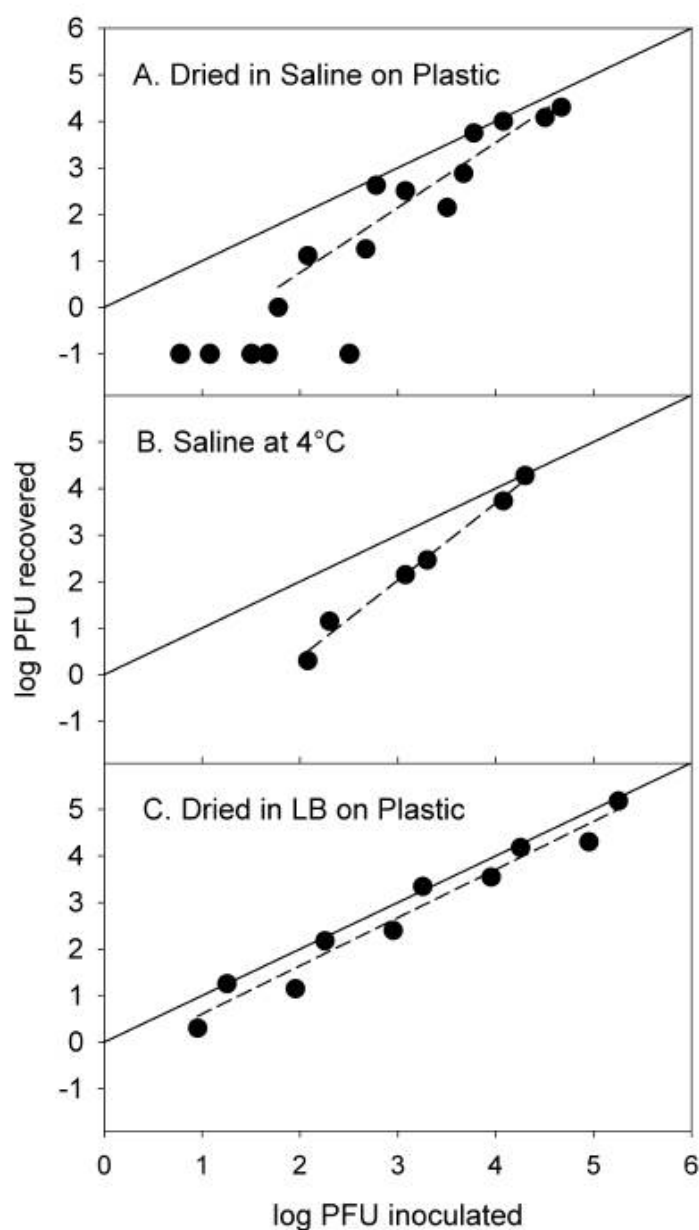


Figure 1