

1 Heat-treated virus inactivation rate depends strongly on
2 treatment procedure: illustration with SARS-CoV-2

3 Amandine Gamble^{1,*}, Robert J. Fischer^{2,*}, Dylan H. Morris³, Kwe Claude Yinda², Vincent J.
4 Munster², and James O. Lloyd-Smith¹

5 ¹*Department of Ecology & Evolutionary Biology, University of California, Los Angeles, CA, USA*

6 ²*Laboratory of Virology, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA*

7 ³*Department of Ecology & Evolutionary Biology, Princeton University, NJ, USA*

8 **Amandine Gamble and Robert J. Fischer contributed equally to this work. Author order was determined*
9 *in order of increasing seniority.*

10 Correspondence: Amandine Gamble, amandine.gamble@gmail.com

11 Running title: Heat treatment for virus inactivation

12 **Abstract**

13 Decontamination can limit environmental transmission of infectious agents. It is required
14 for the safe re-use of contaminated medical, laboratory and personal protective equipment,
15 and for the safe handling of biological samples. Heat is widely used for inactivation of
16 infectious agents, notably viruses. We show that for liquid specimens (here, solution of
17 SARS-CoV-2 in cell culture medium), virus inactivation rate under heat treatment at 70°C
18 can vary by almost two orders of magnitude depending on the treatment procedure, from
19 a half-life of 0.86 min (95% credible interval: [0.09, 1.77]) in closed vials in a heat block
20 to 37.00 min ([12.65, 869.82]) in uncovered plates in a dry oven. These findings suggest
21 a critical role of evaporation in virus inactivation via dry heat. Placing samples in open

22 or uncovered containers may dramatically reduce the speed and efficacy of heat treatment
23 for virus inactivation. We conducted a literature review focused on the effect of tempera-
24 ture on coronavirus stability and found that specimen containers, and whether they were
25 closed, covered or uncovered, are rarely reported in the scientific literature. Heat-treatment
26 procedures must be fully specified when reporting experimental studies to facilitate result
27 interpretation and reproducibility, and carefully considered when designing decontamination
28 guidelines.

29 **Importance**

30 Heat is a powerful weapon against most infectious agents. It is widely used for decontamina-
31 tion of medical, laboratory and personal protective equipment, and for biological samples.
32 There are many methods of heat treatment, and methodological details can affect speed
33 and efficacy of decontamination. We applied four different heat-treatment procedures to
34 liquid specimens containing SARS-CoV-2. The results reveal an important effect of the
35 containers used to store specimens during decontamination: for a given initial level of con-
36 tamination, decontamination time can vary from a few minutes in closed vials to several
37 hours in uncovered plates. Reviewing the literature, we found that container choices and
38 heat treatment methods are rarely made explicit in methods sections. Our study shows that
39 careful consideration of heat-treatment procedure—in particular the choice of specimen con-
40 tainer, and whether it is covered—can make results more consistent across studies, improve
41 decontamination practice, and provide insight into the mechanisms of virus inactivation.

42 **Keywords**

43 Environmental stability, environmental persistence, decontamination, temperature, heat
44 treatment, coronavirus

45 Introduction

46 The COVID-19 pandemic has led to millions of infections worldwide via multiple modes of
47 transmission. Transmission is thought to occur via respiratory particles expelled by indi-
48 viduals infected by the causative virus, SARS-CoV-2 [1–3]. Epidemiological investigations
49 suggest the occurrence of environmental transmission of SARS-CoV-2 [4], which is possible
50 because the virus remains stable for a period of time on inert surfaces and in aerosols [5, 6].
51 Environmental transmission has been suspected or demonstrated for many other viruses, in-
52 cluding hepatitis viruses [7], noroviruses [8], and influenza viruses [9] among others. Rapid
53 and effective decontamination methods can help limit environmental transmission during
54 infectious disease outbreaks.

55 Heat is widely used for the inactivation of various infectious agents, notably viruses [10]. It
56 is thought to inactivate viruses mainly by denaturing the secondary structures of proteins
57 and other molecules, resulting in impaired function [11]. It is used for the decontamination
58 of various materials, such as personal protective equipment (PPE), examination and surgical
59 tools, culture and transportation media, and biological samples [12–15]. For SARS-CoV-2,
60 the United States Centers for Disease Control and Prevention recommends moist heat as a
61 virus inactivation method [16].

62 In this context, studies have evaluated the effectiveness of heat to inactivate coronaviruses
63 on various household surfaces, PPE, culture and transportation media, and blood products
64 [14, 17–22]. Heat-based decontamination procedures are also used for many other viruses,
65 including hepatitis viruses [23], influenza viruses [24], parvoviruses [25], and human immun-
66 odeficiency viruses [26].

67 There are multiple ways to apply heat treatment. Heat can be dry or moist, heating imple-
68 ments can differ in degree of heat transfer (e.g. oven versus heat block, the latter theoret-
69 ically allowing a higher heat transfer), and different levels of evaporation may be permitted
70 (e.g. samples deposited on flat surfaces or contained in open plates will evaporate more than

71 those in closed vials; both types of container are used in laboratories). Local temperature
72 and humidity impact virus inactivation rates by affecting molecular interactions and solute
73 concentration [27]. It follows that factors such as heat transfer and evaporation (which de-
74 termine solute concentration and alter microenvironment temperature through evaporative
75 cooling) likely modulate virus inactivation rates just as ambient temperature does.

76 We assessed the impact of heat-treatment procedure on SARS-CoV-2 inactivation. We
77 studied dry heat treatment applied to a liquid specimen (virus suspension in cell culture
78 medium), keeping temperature constant (at 70°C) but allowing different degrees of heat
79 transfer (using a dry oven or a heat block) and evaporation (placing samples in closed
80 vials, covered plates or uncovered plates). We then compared the half-lives of SARS-CoV-2
81 under these different procedures. In light of our results, we reviewed the literature to assess
82 whether heat-treatment procedure descriptions are detailed enough to allow replication and
83 inter-study comparison. We focused our literature review on coronavirus inactivation.

84 **Results**

85 **Estimation of SARS-CoV-2 half-life under four distinct heat-treatment** 86 **procedures**

87 We prepared a solution of cell culture medium containing SARS-CoV-2, and exposed it to
88 70°C heat following four procedures using different sample containers or heating systems:
89 (1) an uncovered 24-well plate, (2) a covered 24-well plate (using an unsealed plastic lid),
90 (3) a set of closed 2 mL vials in a dry oven, and (4) a set of closed 2 mL vials in a
91 heat block containing water. Three replicates were performed for each treatment. The
92 inactivation rate of SARS-CoV-2 differed sharply across the heat-treatment procedures.
93 There were large differences in the time until the virus dropped below detectable levels
94 despite comparable initial quantities of virus (estimated mean initial titer ranging from
95 4.5 [4.1, 5.0] \log_{10} TCID₅₀/mL for the uncovered plate in an oven to 5.0 [4.7, 5.5] for the

96 closed vials in a heat block, Fig. 1). We could not detect viable virus in the medium after
97 30 min of treatment (the earliest time-point) in closed vials heated either in a heat block or
98 in a dry oven; we could not detect viable virus after 90 min in covered plates (Fig. 1). In
99 uncovered plates, we observed a reduction of viral titer of approximately $1 \log_{10}$ TCID₅₀/mL
100 after 60 min. Macroscopic evaporation was observed in the uncovered plates and almost
101 complete at 60 min.

102 Using a Bayesian regression model, we estimated inactivation rates from the experimental
103 data and converted them to half-lives to compare the four procedures. SARS-CoV-2 inac-
104 tivation in the solution was most rapid in closed vials, using either a heat block or a dry
105 oven (half-lives of 0.86 [0.09, 1.77] and 1.91 [0.10, 1.99] min, respectively), compared to the
106 other treatment procedures (Fig. 2; Supplemental Material, Table S1). Inactivation rate
107 was intermediate in covered plates (half-life of 3.94 [3.12, 5.01] min) and considerably slower
108 in uncovered plates (37.04 [12.65, 869.82] min).

109 Reporting of heat-treatment procedures in the literature

110 Given these findings, we conducted a literature review in order to assess whether heat-
111 treatment procedures were reported with sufficient details to allow reproducibility. Our
112 literature review identified 41 studies reporting the effect of temperature on coronavirus
113 stability (Fig. S1), covering 12 coronavirus species, and temperature ranging from -70 to
114 100°C (Table. S2). Among those 41 studies, 14 included some information about the con-
115 tainers used, and 5 specified whether containers were closed. Only a single study reported
116 container type and container closure explicitly for all experimental conditions [28]. Studies
117 of virus stability in bulk liquid medium were conducted in vials [6, 20, 21, 28–35]. Studies
118 interested in virus stability on surfaces were conducted directly in vials, in well plates [36] or
119 trays [37], or on surface coupons placed in vials [29] or placed directly on oven rack (personal
120 communication [14]). When specified, vial volume ranged from 1.5 mL to 50 mL [21, 30,
121 32], and sample volume from 0.001 to 45 mL. Finally, 24 studies included some information

122 about how controlled temperature (and, in some cases, humidity) conditions were created.
123 Methods included water baths [17, 19, 20, 28, 32, 34, 35, 38, 39], heat blocks [30, 33, 40],
124 incubators [37, 41–45], ovens [14], refrigerators [46–48], isothermal boxes [47], and boxes
125 with saturated salt solutions [49].

126 Discussion

127 Using SARS-CoV-2 as an illustration, we demonstrate that the choice of heat treatment
128 procedure has a considerable impact on virus inactivation in liquid specimens. In liquid
129 specimens (here, virus suspension in cell culture medium), SARS-CoV-2 half-life can vary
130 from 0.86 min ([0.09, 1.77]) in closed vials to 37.0 min ([12.65, 869.82]) in uncovered plates
131 treated with dry heat at 70°C. The rapid virus inactivation rate seen in closed vials subject
132 to dry heat at 70°C agrees with previously reported results for inactivation of SARS-CoV-2
133 in virus transportation medium [50], SARS-CoV-1 in human serum [17], and MERS-CoV
134 [18] and canine coronavirus in cell culture medium [21], among other results. All showed a
135 loss of infectivity on the order of 10^{4-6} TCID₅₀ after 5–10 min at 65–75°C. None of these
136 studies report sufficient details on their protocol to know which of our tested procedures
137 corresponds most closely to their approach.

138 Our findings suggest that evaporation may play a critical role in determining the rate of virus
139 inactivation during dry heat treatment. There are several mechanisms by which evaporation
140 could impact the effectiveness of heat treatments to inactivate viruses. First, evaporation
141 could induce a local drop in temperature due to the enthalpy of vaporization of water (or
142 evaporative cooling), limiting the effect of heat itself. Second, evaporation could lead to
143 modifications of the virion’s solute environment: solutes become more concentrated as the
144 solvent evaporates, and under certain conditions efflorescence (i.e., crystal formation) can
145 occur [51]. Mechanistic modeling of virus inactivation data shows that increased solute
146 concentration increases virus inactivation rate, but efflorescence decreases inactivation rate
147 [27]. We find that the more evaporation is allowed during dry heat treatment, the slower

148 inactivation becomes. This suggests that evaporative cooling, efflorescence, or both, but
149 not concentration of dissolved solutes, may drive lower inactivation rates in closed contain-
150 ers. This could help explain why low ambient humidity levels lead to slow inactivation
151 at high temperatures [43], as low humidity levels allow for more evaporation and possibly
152 efflorescence.

153 Better understanding the impact of temperature and humidity on virus inactivation is crit-
154 ical not only for designing efficient decontamination protocols but also for predicting virus
155 persistence under different environmental conditions, with consequences for real-world trans-
156 mission [27, 52, 53]. Heat transfer could potentially also play a role in determining the rate
157 of virus inactivation using dry heat, but our experimental design did not allow us to explore
158 this hypothesis since virus inactivation was extremely rapid in closed vials regardless of
159 whether they were exposed to heat using an dry oven or a heat block.

160 Given the substantial effect of heat-treatment procedure on virus inactivation rates, it is
161 critical to specify procedures precisely when comparing inactivation rates between studies
162 or producing guidelines for decontamination. In particular, our results show that protocols
163 that use open containers or uncovered surfaces lead to much slower viral inactivation, at
164 least in bulk medium. If meta-analyses of the effect of temperature on virus inactivation
165 were to integrate together data collected following different procedures, without corrections,
166 they may lead to false conclusions.

167 Our work has critical implications for practical decontamination practice using heat treat-
168 ment. Inactivation rates reported by studies conducted using closed vials may dramatically
169 underestimate the time needed to decontaminate a piece of equipment (uncovered) in a dry
170 oven. We have previously estimated the half-life of SARS-CoV-2 on stainless steel and N95
171 fabric when exposed to 70°C using a dry oven, without a container to limit evaporation.
172 We found half-lives of approximately 9 and 5 min, respectively [14]. These values are on
173 the same order of magnitude as the half-life of the virus in bulk solution exposed to heat
174 treatment in a covered plate (3.94 [3.12, 5.01] min), and considerably higher than the half-

175 life of the virus exposed to heat treatment in bulk solution in a closed vial. Inactivation
176 rates reported by studies conducted on closed vials should not be used to directly inform
177 decontamination guidelines of pieces of equipment that cannot be treated using the same
178 exact procedure.

179 Despite the limited information available, our literature review reveals that a variety of se-
180 tups are used to hold samples and control environmental conditions for virus stability and
181 inactivation experiments. Unfortunately, the majority of studies of heat treatment for virus
182 inactivation do not report the exact procedures under which the samples were exposed to
183 heat (in particular whether they were in closed, covered, or uncovered containers). This
184 makes it difficult to compare inactivation rates among studies, and risky to use estimates
185 from the literature to inform decontamination guidelines. More generally, given the large
186 effect of environment on virus inactivation rate, we recommend that decontamination pro-
187 cedures be validated specifically for the setup to be used, rather than based on inactivation
188 rate estimates from the literature, especially if experimental protocols are unclear.

189 Our study focuses exclusively on virus inactivation by heat. Other factors may affect virus
190 inactivation rate in liquid specimens, including pH, salinity, and protein concentration [20,
191 51, 54]; we consider these only implicitly, insofar as they are affected by evaporation. In
192 addition, the impact of heat treatment procedure on inactivation rate may differ across mi-
193 crobes. Enveloped and non-enveloped viruses may behave differently from each other, and
194 bacteria may behave differently from viruses [51]. Finally, decontamination procedures must
195 consider not only the effectiveness and speed of pathogen inactivation but also the potential
196 impact of the procedure on the integrity of the decontaminated equipment or specimen.
197 This is particularly important for PPE and for biological samples [14, 55, 56]. Any effort to
198 translate inactivation rates (or even relative patterns) from one setting to another should
199 thus be undertaken cautiously, accounting for these factors. Effective, reliable decontam-
200 ination requires careful attention to treatment procedure; results from the literature with
201 unclear methods may not be translatable.

202 **Material and Methods**

203 **Laboratory experiments**

204 We used SARS-CoV-2 strain HCoV-19 nCoV-WA1-2020 (MN985325.1) [57] for all our ex-
205 periments. We prepared a solution of SARS-CoV-2 in Dulbecco's Modified Eagle Medium
206 cell culture medium (Sigma-Aldrich, reference D6546) supplemented with 2 nM L-glutamine,
207 2% fetal bovine serum and 100 units/mL penicillin/streptomycin. For each of the four heat-
208 treatment procedures considered, we placed samples of 1 mL of this solution in plate wells
209 or vials before heat treatment. Samples were removed at 10, 20, 30 and 60 min from the
210 uncovered 24-well plate, or at 30, 60 and 90 min for the three other procedures. We took
211 a 0 min time-point measurement prior to exposing the specimens to the heat treatment.
212 As evaporation was observed after exposure to heat, all the samples were complemented
213 to 1 mL with suspension medium (supplemented DMEM) at sampling. At each collection
214 time-point, samples were transferred into a vial and frozen at -80°C until titration (or di-
215 rectly frozen for experiments conducted in vials). We performed three replicates for each
216 inactivation procedure. Samples were not exposed to direct sunlight during the experiment.
217 We quantified viable virus contained in the collected samples by end-point titration on Vero
218 E6 cells as described previously [14].

219 **Statistical analyses**

220 We quantified the inactivation rate of SARS-CoV-2 in a solution following different heat-
221 treatment procedures by adapting a Bayesian approach described previously [14]. Briefly,
222 we inferred virus titers from raw endpoint titration well data by modeling well infections as
223 a Poisson single-hit process [58]. Then, we estimated the decay rates of viable virus titer
224 using a regression model. This modeling approach allowed us to account for differences in
225 initial virus titers (0 min time-point) across samples as well as other sources of experimental
226 noise. The model yields posterior distributions for the virus inactivation rate under each
227 of the treatment procedures—that is, estimates of the range of plausible values for each of

228 these parameters given our data, with an estimate of the overall uncertainty [59]. We then
229 calculated half-lives from the estimated inactivation rates. We analyzed data obtained under
230 different treatment procedures separately. We placed weakly informative prior distributions
231 on mean initial virus titers and log virus half-lives. The complete model is detailed in the
232 [Supplemental Material](#).

233 We estimated virus titers and model parameters by drawing posterior samples using Stan
234 [60], which implements a No-U-Turn Sampler (a form of Markov Chain Monte Carlo), via
235 its R interface RStan. We report estimated titers and model parameters as the median [95%
236 credible interval] of their posterior distribution. We assessed convergence by examining trace
237 plots and confirming sufficient effective sample sizes and \hat{R} values for all parameters. We
238 confirmed appropriateness of prior distributions with prior predictive checks and assessed
239 goodness of fit by plotting regression lines against estimated titers and through posterior
240 predictive (SI, Fig. [S2-S4](#)).

241 Literature review

242 We screened the Web of Science Core Collection database on December 28, 2020 using the
243 following key words: “coronavir* AND (stability OR viability OR inactiv*) AND (temper-
244 ature OR heat OR humidity)” (190 records). We also considered opportunistically found
245 publications (23 records). We then selected the studies reporting original data focused on
246 the effect of temperature on coronavirus inactivation obtained in experimental conditions
247 (Fig. [S1](#)). For each selected study, we recorded information on virus, suspension medium,
248 container, incubator, temperature and humidity (Table. [S2](#)).

249 Data accessibility

250 Code and data to reproduce the Bayesian estimation results and produce corresponding
251 figures are available on Github: <https://github.com/dylanharris/heat-inactivation>
252 [[61](#)]

253 Supplemental Material file list

254 Supplemental file 1 - Supplemental text, tables (Tables S1-S2) and figures (Figures S1-S4).
255 PDF file

256 Acknowledgments

257 We thank Linsey C. Marr for helpful discussions. This research was supported by the
258 Intramural Research Program of the National Institute of Allergy and Infectious Diseases
259 (NIAID), National Institutes of Health (NIH). JOL-S, AG, and DHM were supported by the
260 Defense Advanced Research Projects Agency DARPA PREEMPT (D18AC00031), JOL-S
261 and AG were supported by the UCLA AIDS Institute and Charity Treks, and JOL-S was
262 supported by the U.S. National Science Foundation (DEB-1557022), the Strategic Environ-
263 mental Research and Development Program (SERDP, RC-2635) of the U.S. Department of
264 Defense.

265 References

- 266 1. Ong, S. W. X. *et al.* Air, Surface Environmental, and Personal Protective Equipment
267 Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
268 from a Symptomatic Patient. *JAMA* (2020).
- 269 2. Cai, J. *et al.* Indirect Virus Transmission in Cluster of COVID-19 Cases, Wenzhou,
270 China, 2020. *Emerging Infectious Diseases* **26** (2020).
- 271 3. Kwon, K.-S. *et al.* Evidence of Long-Distance Droplet Transmission of SARS-CoV-2
272 by Direct Air Flow in a Restaurant in Korea. *Journal of Korean medical science* **35**
273 (2020).
- 274 4. Azimi, P., Keshavarz, Z., Cedeno Laurent, J. G., Stephens, B. & Allen, J. G. Mechanis-
275 tic transmission modeling of COVID-19 on the Diamond Princess cruise ship demon-
276 strates the importance of aerosol transmission. *Proceedings of the National Academy of*

- 277 *Sciences* **118**. eprint: [https://www.pnas.org/content/118/8/e2015482118.full.](https://www.pnas.org/content/118/8/e2015482118.full.pdf)
278 [pdf. https://www.pnas.org/content/118/8/e2015482118](https://www.pnas.org/content/118/8/e2015482118) (2021).
- 279 5. van Doremalen, N. *et al.* Aerosol and Surface Stability of SARS-CoV-2 as Compared
280 with SARS-CoV-1. *New England Journal of Medicine*, NEJMc2004973 (2020).
- 281 6. Matson, M. J. *et al.* Effect of Environmental Conditions on SARS-CoV-2 Stability in
282 Human Nasal Mucus and Sputum. *Emerging Infectious Diseases* **26** (2020).
- 283 7. Pfaender, S. *et al.* Environmental Stability and Infectivity of Hepatitis C Virus (HCV)
284 in Different Human Body Fluids. *Frontiers in microbiology* **9**, 504 (2018).
- 285 8. Lopman, B. *et al.* Environmental Transmission of Norovirus Gastroenteritis. *Current*
286 *opinion in virology* **2**, 96–102 (2012).
- 287 9. Breban, R., Drake, J. M., Stallknecht, D. E. & Rohani, P. The Role of Environmental
288 Transmission in Recurrent Avian Influenza Epidemics. *PLoS computational biology* **5**,
289 e1000346 (2009).
- 290 10. Rogers, W. in *Sterilisation of Biomaterials and Medical Devices* 20–55 (Elsevier, 2012).
- 291 11. Wigginton, K. R., Pecson, B. M., Sigstam, T., Bosshard, F. & Kohn, T. Virus Inactiva-
292 tion Mechanisms: Impact of Disinfectants on Virus Function and Structural Integrity.
293 *Environmental Science & Technology* **46**, 12069–12078 (2012).
- 294 12. Chang, L., Yan, Y. & Wang, L. Coronavirus Disease 2019: Coronaviruses and Blood
295 Safety. *Transfusion Medicine Reviews* (2020).
- 296 13. Henwood, A. F. Coronavirus Disinfection in Histopathology. *Journal of Histotechnology*
297 (2020).
- 298 14. Fischer, R. J. *et al.* Effectiveness of N95 Respirator Decontamination and Reuse against
299 SARS-CoV-2 Virus. *Emerging Infectious Diseases* **26**, 2253 (2020).
- 300 15. Heimbuch, B. K. *et al.* A Pandemic Influenza Preparedness Study: Use of Energetic
301 Methods to Decontaminate Filtering Facepiece Respirators Contaminated with H1N1
302 Aerosols and Droplets. *American Journal of Infection Control* **39**, 265–270 (2011).

- 303 16. Centers for Disease Control and Prevention. *Implementing Filtering Facepiece Respi-*
304 *rator (FFR) Reuse, Including Reuse after Decontamination, When There Are Known*
305 *Shortages of N95 Respirators* 2020.
- 306 17. Darnell, M. E. R. & Taylor, D. R. Evaluation of Inactivation Methods for Severe
307 Acute Respiratory Syndrome Coronavirus in Noncellular Blood Products. *Transfusion*
308 **46**, 1770–1777 (2006).
- 309 18. Leclercq, I., Batéjat, C., Burguière, A. M. & Manuguerra, J.-C. Heat Inactivation of
310 the Middle East Respiratory Syndrome Coronavirus. *Influenza and Other Respiratory*
311 *Viruses* **8** (2014).
- 312 19. Pagat, A.-M. *et al.* Evaluation of SARS-Coronavirus Decontamination Procedures.
313 *Applied Biosafety* **12**, 100–108 (2007).
- 314 20. Laude, H. Thermal Inactivation Studies of a Coronavirus, Transmissible Gastroenteritis
315 Virus. *The Journal of General Virology* **56**, 235–240 (1981).
- 316 21. Pratelli, A. Canine Coronavirus Inactivation with Physical and Chemical Agents. *The*
317 *Veterinary Journal* **177**, 71–79 (2008).
- 318 22. Duan, S.-M. *et al.* Stability of SARS Coronavirus in Human Specimens and Environ-
319 *ment and Its Sensitivity to Heating and UV Irradiation. Biomedical and Environmental*
320 *Sciences* **16**, 246–255 (2003).
- 321 23. Abu-Aisha, H. *et al.* The Effect of Chemical and Heat Disinfection of the Hemodialysis
322 *Machines on the Spread of Hepatitis c Virus Infection: A Prospective Study. Saudi*
323 *Journal of Kidney Diseases and Transplantation* **6**, 174 (1995).
- 324 24. Jeong, E. K., Bae, J. E. & Kim, I. S. Inactivation of Influenza A Virus H1N1 by
325 *Disinfection Process. American Journal of Infection Control* **38**, 354–360 (2010).
- 326 25. Eterpi, M., McDonnell, G. & Thomas, V. Disinfection Efficacy against Parvoviruses
327 *Compared with Reference Viruses. Journal of Hospital Infection* **73**, 64–70 (2009).

- 328 26. World Health Organization. *Guidelines on Sterilization and Disinfection Methods Effective against Human Immunodeficiency Virus (HIV)* tech. rep. (1989).
329
- 330 27. Morris, D. H. *et al.* Mechanistic Theory Predicts the Effects of Temperature and Humidity on Inactivation of SARS-CoV-2 and Other Enveloped Viruses. *bioRxiv*. eprint: <https://www.biorxiv.org/content/early/2020/12/18/2020.10.16.341883.full.pdf> (2020).
331
332
333
- 334 28. Hulst, M. M. *et al.* Study on Inactivation of Porcine Epidemic Diarrhoea Virus, Porcine Sapelovirus 1 and Adenovirus in the Production and Storage of Laboratory Spray-Dried Porcine Plasma. *Journal of Applied Microbiology* **126**, 1931–1943 (2019).
335
336
- 337 29. Chan, K.-H. *et al.* Factors Affecting Stability and Infectivity of SARS-CoV-2. *Journal of Hospital Infection* **106**, 226–231 (2020).
338
- 339 30. Darnell, M. E. R., Subbarao, K., Feinstone, S. M. & Taylor, D. R. Inactivation of the Coronavirus That Induces Severe Acute Respiratory Syndrome, SARS-CoV. *Journal of Virological Methods* **121**, 85–91 (2004).
340
341
- 342 31. Gundy, P. M., Gerba, C. P. & Pepper, I. L. Survival of Coronaviruses in Water and Wastewater. *Food and Environmental Virology* **1** (2009).
343
- 344 32. Kariwa, H., Fujii, N. & Takashima, K. Inactivation of SARS Coronavirus by Means of Povidone-Iodine, Physical Conditions and Chemical Reagents. *Dermatology* **212**, 119–123 (2006).
345
346
- 347 33. Kim, Y.-I. *et al.* Development of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Thermal Inactivation Method with Preservation of Diagnostic Sensitivity. *Journal of Microbiology* **58**, 886–891 (2020).
348
349
- 350 34. Pujols, J. & Segalés, J. Survivability of Porcine Epidemic Diarrhea Virus (PEDV) in Bovine Plasma Submitted to Spray Drying Processing and Held at Different Time by Temperature Storage Conditions. *Veterinary microbiology* **174**, 427–432 (2014).
351
352

- 353 35. Saknimit, M., Inatsuki, I., Sugiyama, Y. & Yagami, K. Virucidal Efficacy of Physico-
354 Chemical Treatments against Coronaviruses and Parvoviruses of Laboratory Animals.
355 *Experimental Animals* **37**, 341–345 (1988).
- 356 36. Chan, K. H. *et al.* The Effects of Temperature and Relative Humidity on the Viability
357 of the SARS Coronavirus. *Advances in Virology* **2011**, 1–7 (2011).
- 358 37. Thomas, P. R. *et al.* Evaluation of Time and Temperature Sufficient to Inactivate
359 Porcine Epidemic Diarrhea Virus in Swine Feces on Metal Surfaces. *Journal of Swine*
360 *Health and Production* **23**, 84 (2015).
- 361 38. Hofmann, M. & Wyler, R. Quantitation, Biological and Physicochemical Properties
362 of Cell Culture-Adapted Porcine Epidemic Diarrhea Coronavirus (PEDV). *Veterinary*
363 *microbiology* **20**, 131–142 (1989).
- 364 39. Unger, S. *et al.* Holder Pasteurization of Donor Breast Milk Can Inactivate SARS-
365 CoV-2. *Canadian Medical Association Journal* **192**, E1657–E1661 (2020).
- 366 40. Batéjat, C., Grassin, Q., Manuguerra, J.-C. & Leclercq, I. Heat Inactivation of the
367 Severe Acute Respiratory Syndrome Coronavirus 2. *bioRxiv*, 2020.05.01.067769 (2020).
- 368 41. van Doremalen, N., Bushmaker, T. & Munster, V. Stability of Middle East Respira-
369 tory Syndrome Coronavirus (MERS-CoV) under Different Environmental Conditions.
370 *Eurosurveillance* **18**, 20590 (2013).
- 371 42. Riddell, S., Goldie, S., Hill, A., Eagles, D. & Drew, T. W. The Effect of Temperature
372 on Persistence of SARS-CoV-2 on Common Surfaces. *Virology Journal* **17** (2020).
- 373 43. Rockey, N. *et al.* Humidity and Deposition Solution Play a Critical Role in Virus
374 Inactivation by Heat Treatment of N95 Respirators. *Mosphere* **5** (2020).
- 375 44. Daeschler, S. C. *et al.* Effect of Moist Heat Reprocessing of N95 Respirators on SARS-
376 CoV-2 Inactivation and Respirator Function. *CMAJ* **192**, E1189–E1197 (2020).
- 377 45. Biryukov, J. *et al.* Increasing Temperature and Relative Humidity Accelerates Inacti-
378 vation of SARS-CoV-2 on Surfaces. *mSphere* **5** (2020).

- 379 46. Mullis, L., Saif, L. J., Zhang, Y., Zhang, X. & Azevedo, M. S. Stability of Bovine
380 Coronavirus on Lettuce Surfaces under Household Refrigeration Conditions. *Food Mi-*
381 *crobiology* **30**, 180–186 (2012).
- 382 47. Guionie, O. *et al.* An Experimental Study of the Survival of Turkey Coronavirus at
383 Room Temperature and +4C. *Avian Pathology* **42**, 248–252 (2013).
- 384 48. Casanova, L., Rutala, W. A., Weber, D. J. & Sobsey, M. D. Survival of Surrogate
385 Coronaviruses in Water. *Water Research* **43**, 1893–1898 (2009).
- 386 49. Casanova, L. M., Jeon, S., Rutala, W. A., Weber, D. J. & Sobsey, M. D. Effects of Air
387 Temperature and Relative Humidity on Coronavirus Survival on Surfaces. *Applied and*
388 *Environmental Microbiology* **76**, 2712–2717 (2010).
- 389 50. Chin, A. W. H. *et al.* Stability of SARS-CoV-2 in Different Environmental Conditions.
390 *The Lancet Microbe* **1**, e10 (2020).
- 391 51. Yang, W. & Marr, L. C. Mechanisms by Which Ambient Humidity May Affect Viruses
392 in Aerosols. *Applied and Environmental Microbiology* **78**, 6781–6788 (2012).
- 393 52. Posada, J., Redrow, J. & Celik, I. A Mathematical Model for Predicting the Viability
394 of Airborne Viruses. *Journal of Virological Methods* **164**, 88–95 (2010).
- 395 53. Lofgren, E., Fefferman, N. H., Naumov, Y. N., Gorski, J. & Naumova, E. N. Influenza
396 Seasonality: Underlying Causes and Modeling Theories. *Journal of Virology* **81**, 5429–
397 5436 (2007).
- 398 54. Benbough, J. E. Some Factors Affecting the Survival of Airborne Viruses. *The Journal*
399 *of General Virology* **10**, 209–220 (1971).
- 400 55. Estep, T. N., Bechtel, M. K., Miller, T. J. & Bagdasarian, A. Virus Inactivation in
401 Hemoglobin Solutions by Heat. *Biomaterials, Artificial Cells and Artificial Organs*
402 **16**, 129–134 (1988).
- 403 56. Gertsman, S. *et al.* Microwave- and Heat-Based Decontamination of N95 Filtering
404 Facepiece Respirators: A Systematic Review. *Open Science Framewor* (2020).

- 405 57. Holshue, M. L. *et al.* First Case of 2019 Novel Coronavirus in the United States. *New*
406 *England Journal of Medicine* **382**, 929–936 (2020).
- 407 58. Brownie, C. *et al.* Estimating Viral Titres in Solutions with Low Viral Loads. *Biologi-*
408 *cals* **39**, 224–230 (2011).
- 409 59. Gelman, A. *et al.* *Bayesian Data Analysis, Third Edition* (CRC Press, 2013).
- 410 60. Carpenter, B. *et al.* Stan: A Probabilistic Programming Language. *Journal of statistical*
411 *software* **76** (2017).
- 412 61. Gamble, A. *et al.* Data from “Heat-treated virus inactivation rate depends strongly on
413 *treatment procedure: illustration with SARS-CoV-2*” [https://github.com/dylanhnmorris/](https://github.com/dylanhnmorris/heat-inactivation)
414 [heat-inactivation](https://github.com/dylanhnmorris/heat-inactivation).
- 415 62. Kay, M., Kola, T., Hullman, J. R. & Munson, S. A. *When (ish) is my bus? user-centered*
416 *visualizations of uncertainty in everyday, mobile predictive systems* in *Proceedings of*
417 *the 2016 CHI Conference on Human Factors in Computing Systems* (2016), 5092–5103.

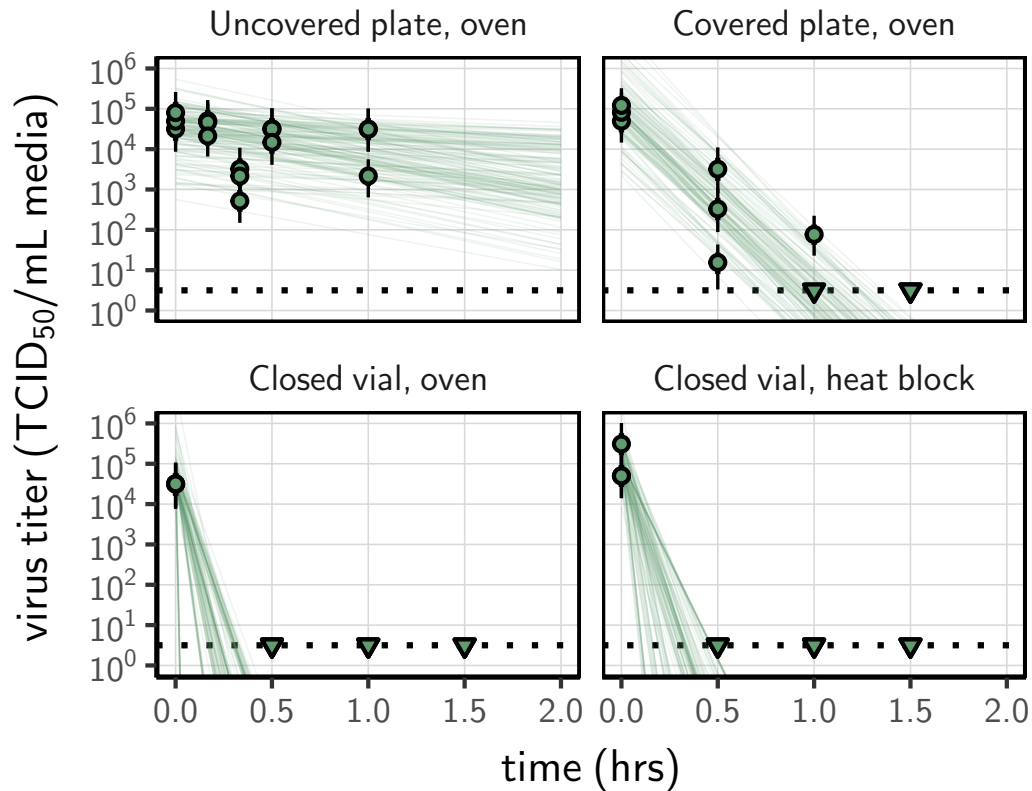


Figure 1. Inactivation of SARS-CoV-2 by heat treatment under different procedures. A solution of SARS-CoV-2 was exposed to 70°C heat. Samples were placed in uncovered or covered 24-well plates, or in closed 2 mL vial before heat treatment using a dry oven or a heat block. Samples were then collected at indicated time-points during heat treatment. Viable virus titer estimated by end-point titration is shown in TCID₅₀/mL media on a logarithmic scale. Points show estimated titers for each collected sample; vertical bar shows a 95% credible interval. Time-points with no positive wells for any replicate are plotted as triangles at the approximate single-replicate detection limit of the assay (LOD; denoted by a black dotted line at 10^{0.5} TCID₅₀/mL media) to indicate that a range of sub-LOD values are plausible. Lines show predicted decay of virus titer over time (10 random draws per datapoint from the joint posterior distribution of the exponential decay rate, i.e. negative of the slope, and datapoint intercept, i.e. initial virus titer).

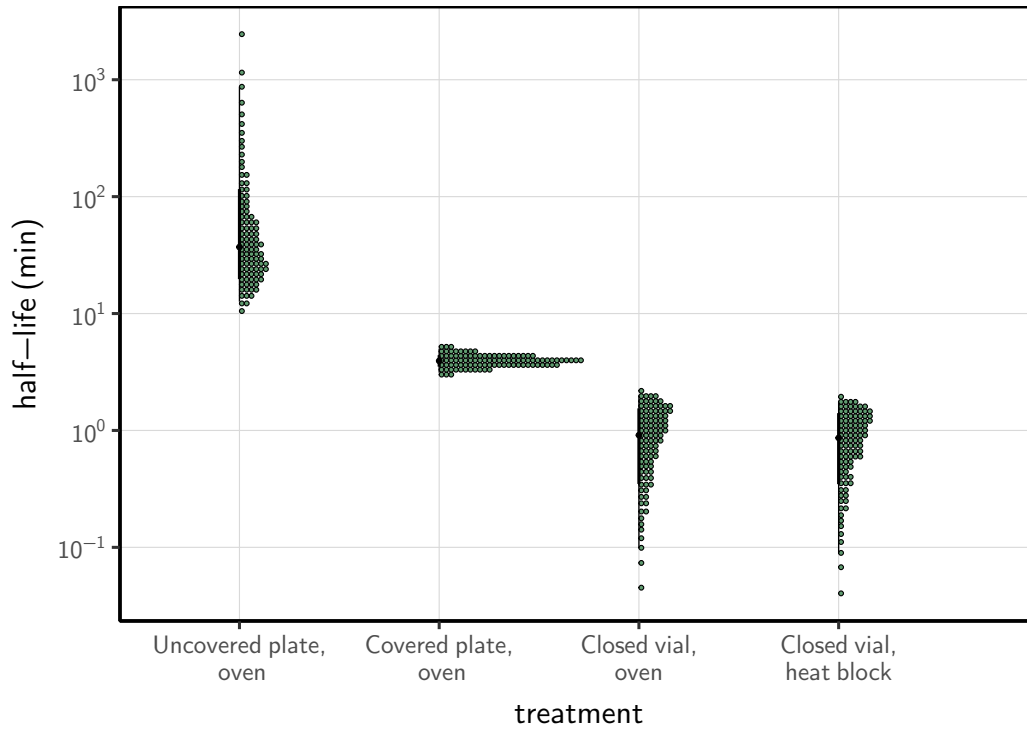


Figure 2. Half-life of SARS-CoV-2 in a solution exposed to 70°C heat under different procedures. Quantile dotplots [62] of the posterior distribution for half-life of viable virus under each different heat-treatment procedure. Half-lives were calculated from the estimated exponential decay rates of virus titer (Fig. 1) and plotted on a logarithmic scale. For each distribution, the black dot shows the posterior median estimate and the black line shows the 95% credible interval.