

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. Munster², and
4 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 in order of increasing
9 seniority.*

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

11 Running title: Heat treatment for virus inactivation

12 **Abstract**

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

24 containers, and whether they are closed, covered, or uncovered, are rarely reported in the
25 scientific literature. Heat-treatment procedures must be fully specified when reporting
26 experimental studies to facilitate result interpretation and reproducibility, and must be carefully
27 considered when developing decontamination guidelines.

28 **Importance**

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

41 **Keywords**

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

44 **Introduction**

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

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

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

65 There are multiple ways to apply heat treatment. Heat can be dry or moist. Heating implements
66 can differ in degree of heat transfer: for example, heat blocks in theory allow more efficient heat

67 transfer than ovens, so samples should more rapidly reach and better maintain the target
68 temperature. Different levels of evaporation may be permitted: for example, samples deposited
69 on flat surfaces or contained in open plates will evaporate more than those in closed vials; both
70 types of container are commonly-used. Local temperature and humidity impact virus inactivation
71 rates by affecting molecular interactions and solute concentration [27]. It follows that factors
72 such as heat transfer and evaporation, which determine solute concentration and alter micro-
73 environment temperature through evaporative cooling, could modulate virus inactivation rates
74 just as ambient temperature does.

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

83 **Results and Discussion**

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

86 We prepared a solution of cell culture medium containing SARS-CoV-2, and exposed it to 70°C
87 heat using four different procedures: (1) an uncovered 24-well plate, (2) a covered 24-well plate
88 (using an unsealed plastic lid), (3) a set of closed 2 mL vials in a dry oven, and (4) a set of closed 2
89 mL vials in a heat block containing water (Fig. 1A). The inactivation rate of SARS-CoV-2 differed

90 sharply across procedures. There were large differences in the time until the virus dropped
91 below detectable levels, despite comparable initial quantities of virus (estimated mean initial
92 titer ranging from 4.5 [4.1, 5.0] \log_{10} TCID₅₀/mL for the uncovered plate in an oven to 5.0 [4.7, 5.5]
93 for the closed vials in a heat block, Fig. 1B). We could not detect viable virus in the medium after
94 30 min of treatment (the earliest time-point) in closed vials heated either in a heat block or in a
95 dry oven; we could not detect viable virus after 90 min in covered plates (Fig. 1B). In uncovered
96 plates, we observed a reduction of viral titer of approximately 1 \log_{10} TCID₅₀/mL after 60 min.
97 Because macroscopic evaporation was observed in the uncovered plates and was almost
98 complete at 60 min, all the samples were complemented to 1 mL with deionized water at
99 collection. Hence, the slower decrease in viral titer observed in uncovered plates (and, to a lesser
100 extent, in covered plates compared to closed vials) can only be explained by a slower inactivation
101 rate, not by virus concentration due to evaporation.

102 Using a Bayesian regression model, we estimated inactivation rates from the experimental data
103 and converted them to half-lives to compare the four procedures. SARS-CoV-2 inactivation in
104 solution was most rapid in closed vials, using either a heat block or a dry oven (half-lives of 0.86
105 [0.09, 1.77] and 1.91 [0.10, 1.99] min, respectively), compared to the other treatment procedures
106 (Fig. 2; [Supplemental Material](#), Table 1). Inactivation rate was intermediate in covered plates
107 (half-life of 3.94 [3.12, 5.01] min) and considerably slower in uncovered plates (37.04 [12.65,
108 869.82] min).

109 The rapid virus inactivation rate seen in closed vials subject to dry heat at 70°C agrees with
110 previously reported results for inactivation of SARS-CoV-2 in virus transportation medium [28],
111 SARS-CoV-1 in human serum [17], and MERS-CoV [18] and canine coronavirus in cell culture
112 medium [21], among other results. All showed a loss of infectivity on the order of 10^{4-6} TCID₅₀

113 after 5–10 min at 65–75°C. None of these studies report sufficient details on their protocol to
114 indicate which of our tested procedures corresponds most closely to their approach.
115 These results have critical implications for real-world heat treatment decontamination practices.
116 Inactivation rates reported in studies that use closed vials may dramatically underestimate the
117 time needed to decontaminate a piece of equipment (uncovered) in a dry oven. We have
118 previously estimated the half-life of SARS-CoV-2 on stainless steel and N95 fabric when exposed
119 to 70°C using a dry oven, without a container to limit evaporation. We found half-lives of
120 approximately 9 and 5 min, respectively [14]. These values are on the same order of magnitude
121 as the half-life of the virus in bulk solution exposed to heat treatment in a covered plate (3.94
122 [3.12, 5.01] min), and considerably longer than the half-life of the virus exposed to heat treatment
123 in bulk solution in a closed vial. Inactivation rates reported by studies conducted in closed vials
124 should not be used to directly inform decontamination guidelines of pieces of equipment that
125 cannot be treated using the same exact procedure.

126 **The potential role of evaporation in virus inactivation**

127 The fact that containers that allow more air-flow are associated with slower virus inactivation
128 suggests that evaporation may play a critical role in determining the rate of virus inactivation
129 during dry heat treatment. There are several mechanisms by which evaporation could impact the
130 effectiveness of heat treatment for virus inactivation. First, evaporation could induce a local drop
131 in temperature due to the enthalpy of vaporization of water (or evaporative cooling), limiting the
132 effect of the heat itself. This hypothesis could be verified in future studies by measuring sample
133 temperature (instead of ambient temperature) using a thermocouple. Second, evaporation could
134 lead to modifications of the virion's solute environment: solutes become more concentrated as
135 the solvent evaporates, and under certain conditions efflorescence (i.e. crystal formation) can
136 occur [29]. Mechanistic modeling of virus inactivation data shows that increased solute

137 concentration increases virus inactivation rate, but efflorescence decreases inactivation rate [27].
138 Our results show that greater degrees of evaporation during dry heat treatment are associated
139 with slower virus inactivation. This suggests that evaporative cooling, efflorescence, or both may
140 drive lower inactivation rates in closed containers. This could help explain why low ambient
141 humidity levels lead to slow inactivation at high temperatures [30], as low humidity levels allow
142 for more evaporation and possibly efflorescence. The potential role of evaporation as a key
143 modulator of virus inactivation rate is supported by the known importance of other factors that
144 affect evaporation, such as relative humidity [27, 29] and medium composition [20, 31]. We
145 postulate that container shape and surface area to volume ratio will also play a role, as these
146 should also impact the evaporative dynamics. Heat transfer efficiency may also play a role in
147 determining the rate of virus inactivation using dry heat, but our data do not provide evidence for
148 or against this hypothesis, since virus inactivation was extremely rapid in closed vials regardless
149 of whether they were exposed to heat using a dry oven or a heat block.
150 Our study focuses exclusively on the effect of temperature on virus inactivation. Other factors can
151 affect virus inactivation rate in liquid specimens, for example the composition of the suspension
152 medium [20, 29, 32]. In particular, proteins are thought to have a protective effect on virus
153 viability, while the effect of salts and pH depends on other factors such as ambient humidity [33].
154 We consider these only implicitly, insofar as they are affected by evaporation. The role of medium
155 composition will be especially important to consider in future studies, as the composition of
156 biological fluids, usually targeted by decontamination procedures, differs greatly from that of cell
157 culture media. In addition, the impact of heat treatment procedure on inactivation rate may differ
158 across microbes. Enveloped and non-enveloped viruses may behave differently from each other,
159 and bacteria may behave differently from viruses [29]. In particular, non-enveloped viruses are
160 generally more stable than enveloped viruses [34], but very few studies have focused on thermal

161 sensitivity [35]. Finally, decontamination procedures must consider not only the effectiveness
162 and speed of pathogen inactivation but also the potential impact of the procedure on the integrity
163 of the decontaminated equipment or specimen. This is particularly important for PPE and for
164 biological samples [14, 36, 37].

165 Given the substantial effect of heat-treatment procedure on virus inactivation rates, it is critical to
166 specify procedures precisely when comparing inactivation rates between studies or producing
167 guidelines for decontamination. In particular, our results show that protocols that use open
168 containers or uncovered surfaces lead to much slower viral inactivation, at least in bulk medium.
169 For instance, the fact that Chin et al. 2020 [28] used closed vials to quantify SARS-CoV-2 half-life
170 (personal communications) likely gave rise to outliers observed at 56 and 70°C relative to
171 predicted relationships parameterized from uncovered surfaces [27]. If meta-analyses of the
172 effect of temperature on virus inactivation were to integrate together data collected following
173 different procedures, without corrections, they may lead to false conclusions.

174 **Reporting of heat-treatment procedures in the literature**

175 Given these findings, we conducted a literature review in order to assess whether heat treatment
176 procedures for coronaviruses were reported with sufficient details to allow reproducibility and
177 appropriate interpretation of results. Our literature review identified 41 studies reporting the
178 effect of temperature on coronavirus stability (Fig. S1), covering 12 coronavirus species and
179 temperature ranging from -70 to 100°C (Table. S1). Among those 41 studies, just 14 included any
180 information about the containers used, and 5 specified whether containers were closed. Only a
181 single study reported container type and container closure explicitly for all experimental
182 conditions [38]. When the information was available, studies of virus stability in bulk liquid
183 medium were always conducted in vials [6, 20, 21, 38–45]. Studies interested in virus stability on
184 surfaces were conducted in vials, in well plates [46] or trays [47], or on surface coupons placed in

185 vials [39] or placed directly on oven rack (personal communication [14]). When specified, vial
186 volume ranged from 1.5 mL to 50 mL [21, 40, 42], and sample volume from 0.001 to 45 mL.
187 Finally, 24 studies included some information about how target temperature (and, in some cases,
188 humidity) conditions were created. Methods included water baths [17, 19, 20, 38, 42, 44, 45, 48,
189 49], heat blocks [40, 43, 50], incubators [30, 47, 51–54], ovens [14], refrigerators [55–57],
190 isothermal boxes [56], and boxes with saturated salt solutions [58].

191 This literature review reveals that a variety of setups are used to hold samples and control
192 environmental conditions for virus stability and inactivation experiments. Unfortunately, it also
193 reveals that the vast majority of studies of heat treatment for virus inactivation do not report the
194 exact procedures under which the samples were exposed to heat (in particular whether they
195 were in closed, covered, or uncovered containers). This makes it difficult to compare inactivation
196 rates among studies, and risky to use estimates from the literature to inform decontamination
197 guidelines. More generally, given the potentially large effects of treatment procedure and
198 ambient environment on virus inactivation rate, we recommend that decontamination
199 procedures be validated specifically for the setup to be used, rather than based on inactivation
200 rate estimates from the literature, especially if experimental protocols are unclear.

201 **Conclusion and Perspectives**

202 Using SARS-CoV-2 as an illustration, we demonstrate that the choice of heat-treatment procedure
203 has a considerable impact on virus inactivation rates in liquid specimens. Our findings highlight
204 the need to better understand the mechanisms controlling inactivation rate, including the role of
205 evaporation. This will require comparative studies including a set of diverse microbes exposed to
206 heat treatment in different conditions likely to impact evaporation dynamics and/or microbe
207 thermal stability, ideally paired with high-resolution physical measurements. These conditions

208 include container sealing, but also sample volume and evaporation surface, medium composition,
209 container material, and heating system. In the meantime, any effort to compare or translate
210 inactivation rates (or even relative patterns) from one setting to another should be undertaken
211 cautiously, accounting for these factors. In particular, as decontamination time can vary by
212 several orders of magnitude across procedures, these factors should be considered when
213 developing decontamination guidelines. Finally, we also call for more thorough description of
214 experimental protocols in scientific publications, for instance through the publication of detailed
215 protocols in online repositories [59], or peer-reviewed journals publishing laboratory protocols.
216 Better understanding the impact of temperature and humidity on virus inactivation is critical not
217 only for designing efficient decontamination protocols but also for predicting virus
218 environmental persistence, with consequences for real-world transmission [27, 60, 61].

219 **Material and Methods**

220 **Laboratory experiments**

221 We used SARS-CoV-2 strain HCoV-19 nCoV-WA1-2020 (MN985325.1) [62] for all our
222 experiments. We prepared a solution of SARS-CoV-2 in Dulbecco's Modified Eagle Medium cell
223 culture medium (Sigma-Aldrich, reference D6546) supplemented with 2 nM L-glutamine, 2%
224 fetal bovine serum and 100 units/mL penicillin/streptomycin. For each of the four heat-
225 treatment procedures considered, we placed samples of 1 mL of this solution in plate wells or
226 vials before heat treatment. This relatively low volume was chosen to allow the samples to reach
227 70°C quickly. The plates were 24-well flat-bottom plates made of crystalline polystyrene with an
228 inner diameter of 15.6 mm (Corning Costar), and 2 mL screw-top vials made of polypropylene
229 with an inner diameter of 10.8 mm diameter (Sarstedt). Both materials have a similar thermal
230 conductivity (0.1-0.13 and 0.1-0.22, respectively, at 23°C) and thickness (about 0.05 mm). The

231 plates and tubes were then placed into either a gene hybridization dry oven or a heat block with
232 water in the wells (Fig. 1A). The large rotating ferris wheel-like apparatus of the gene
233 hybridization oven ensured air mixing during the experiments, preventing a build-up of humid
234 air above the open wells.

235 Samples were removed at 10, 20, 30 and 60 min from the uncovered 24-well plate, or at 30, 60
236 and 90 min for the three other procedures. We took a 0 min time-point measurement prior to
237 exposing the specimens to the heat treatment. As evaporation was observed after exposure to
238 heat, all the samples were complemented to 1 mL with deionized water at collection in order to
239 re-hydrate the suspension medium and recover virions with the same efficiency across all
240 treatments. At each collection time-point, samples were transferred into a vial and frozen at -80°C
241 until titration (or directly frozen for experiments conducted in vials). Note that all the samples
242 were kept frozen for 8 days and subject to one freeze-thaw cycle, which may have some (limited)
243 impact on absolute virus titer [63, 64], but not on the estimated inactivation rate (since this
244 depends on relative titers across samples). We performed three replicates for each inactivation
245 procedure. Samples were not exposed to direct sunlight during the experiment.

246 We quantified viable virus contained in the collected samples by end-point titration as described
247 previously [14]. Briefly, Vero E6 cells were plated the day before carrying out titration. After 24
248 hours, the cells had reached a confluency of about 85-90% and were inoculated with 10-fold
249 serial dilutions of sample in quadruplicates. One hour after inoculation, inoculum was removed
250 and replaced with 100µL of supplemented DMEM. Six days after inoculation, each well was
251 observed for cytopathogenic effects and classified as infected or non-infected.

252 **Statistical analyses**

253 We quantified the inactivation rate of SARS-CoV-2 in a solution following different heat-treatment
254 procedures by adapting a Bayesian approach described previously [14]. Briefly, we inferred virus
255 titers from raw endpoint titration well data (infected / non-infected) by modeling well infections
256 as a Poisson single-hit process [65]. Then, we estimated the decay rates of viable virus titer using
257 a regression model. This modeling approach allowed us to account for differences in initial virus
258 titers (0 min time-point) across samples as well as other sources of experimental noise. The
259 model yields posterior distributions for the virus inactivation rate under each of the treatment
260 procedures—that is, estimates of the range of plausible values for each of these parameters given
261 our data, with an estimate of the overall uncertainty [66]. We then calculated half-lives from the
262 estimated inactivation rates. We analyzed data obtained under different treatment procedures
263 separately. We placed weakly informative prior distributions on mean initial virus titers and log
264 virus half-lives. The complete model is detailed in the [Supplemental Material](#).

265 We estimated virus titers and model parameters by drawing posterior samples using Stan [67],
266 which implements a No-U-Turn Sampler (a form of Markov Chain Monte Carlo), via its R interface
267 RStan. We report estimated titers and model parameters as the median [95% credible interval] of
268 their posterior distribution. We assessed convergence by examining trace plots and confirming
269 sufficient effective sample sizes and \hat{R} values for all parameters. We confirmed appropriateness
270 of prior distributions with prior predictive checks and assessed goodness of fit by plotting
271 regression lines against estimated titers and through posterior predictive checks (SI, Fig. [S2-S4](#)).

272 **Literature review**

273 We screened the Web of Science Core Collection database on December 28, 2020 using the
274 following key words: “coronavir* AND (stability OR viability OR inactiv*) AND (temperature OR

275 heat OR humidity)” (190 records). We also considered opportunistically found publications (23
276 records). We then selected the studies reporting original data focused on the effect of
277 temperature on coronavirus inactivation obtained in experimental conditions (Fig. S1). For each
278 selected study, we recorded information on virus, suspension medium, container, incubator,
279 temperature and humidity (Table S1).

280 **Data accessibility**

281 Compiled literature data as well as code and data to reproduce the Bayesian estimation results
282 and corresponding figures are available on Github: [https://github.com/dylanhmorris/heat-](https://github.com/dylanhmorris/heat-inactivation)
283 [inactivation](https://github.com/dylanhmorris/heat-inactivation) [68]

284 **Supplemental Material file list**

285 Supplemental file 1 - Supplemental text, tables (Table S1) and figures (Figures S1-S4). PDF
286 file

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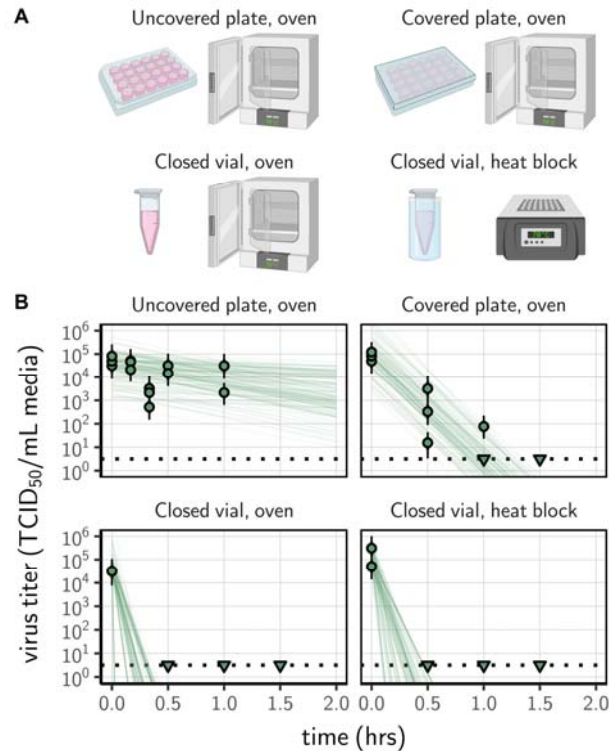
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460 **Table 1.** Half-life of SARS-CoV-2 in Dulbecco's Modified Eagle Medium cell culture medium
461 exposed to 70°C heat under different procedures. Half-lives are calculated from the estimated
462 exponential decay rates of virus titer and reported as posterior median and middle 95% credible
463 interval.

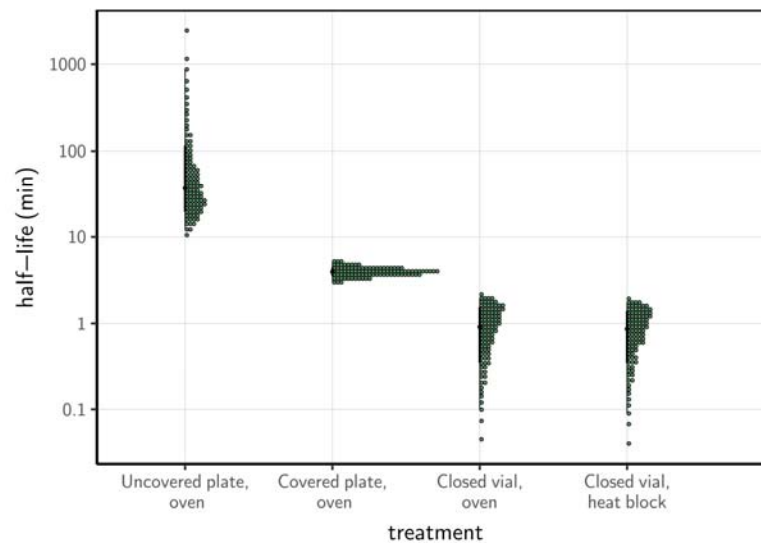
Procedure	Median (min)	2.5%	97.5%
Uncovered plate, oven	37.04	12.65	869.82
Covered plate, oven	3.94	3.12	5.01
Closed vial, oven	0.91	0.10	1.99
Closed vial, heat block	0.86	0.09	1.77

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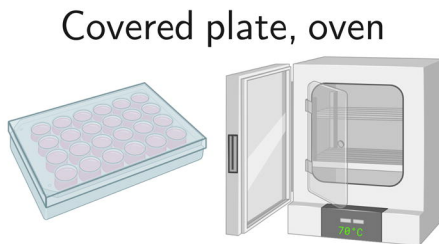
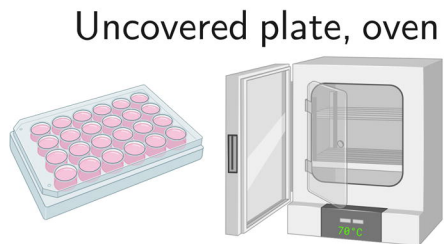
465

466 **Figure 1.** Inactivation of SARS-CoV-2 by heat treatment under different procedures. (A) A
467 solution of SARS-CoV-2 was exposed to 70°C heat. Samples were placed in uncovered or covered
468 24-well plates, or in closed 2 mL vial before heat treatment using a dry oven or a heat block
469 containing water. (B) Samples were then collected at indicated time-points during heat treatment.
470 Viable virus titer estimated by end-point titration is shown in TCID₅₀/mL media on a logarithmic
471 scale. Points show estimated titers for each collected sample; vertical bar shows a 95% credible
472 interval. Time-points with no positive wells for any replicate are plotted as triangles at the
473 approximate single-replicate detection limit of the assay (LOD; denoted by a black dotted line at
474 10^{0.5} TCID₅₀/mL media) to indicate that a range of sub-LOD values are plausible. Lines show
475 predicted decay of virus titer over time (10 random draws per data-point from the joint posterior
476 distribution of the slope and intercept). Panel A created with [BioRender.com](https://www.biorender.com)



477

478 **Figure 2.** Half-life of SARS-CoV-2 in a solution exposed to 70°C heat under different procedures.
479 Quantile dotplots [69] of the posterior distribution for half-life of viable virus under each different
480 heat-treatment procedure. Half-lives were calculated from the estimated exponential decay rates
481 of virus titer (Fig. 1B) and plotted on a logarithmic scale. For each distribution, the black dot
482 shows the posterior median estimate and the black line shows the 95% credible interval.

A**B**