Inactivation of SARS-CoV-2 on surfaces and in solution with Virusend (TX-10), a novel disinfectant

Enyia R. Anderson[1], Grant L. Hughes[1*], Edward I. Patterson[1*]

[1] Departments of Vector Biology and Tropical Disease Biology, Centre for Neglected Tropical Disease, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK

*** these authors contributed equally.**

Correspondence to: ian.patterson@lstmed.ac.uk; grant.hughes@lstmed.ac.uk

Abstract

Until an effective vaccine against SARS-CoV-2 is available on a widespread scale, the control of the COVID-19 pandemic is reliant upon effective pandemic control measures. The ability of SARS-CoV-2 to remain viable on surfaces and in aerosols, means indirect contact transmission can occur and so there is an opportunity to reduce transmission using effective disinfectants in public and communal spaces. Virusend (TX-10), a novel disinfectant, has been developed as a highly effective disinfectant against a range of microbial agents. Here we investigate the ability of Virusend (TX-10) to inactivation SARS-CoV-2. Using surface and solution inactivation assays, we show that Virusend (TX-10) is able to reduce SARS-CoV-2 viral titre by $4\log_{10}$ PFU/mL within 1 minute of contact. Ensuring disinfectants are highly effective against SARS-CoV-2 is important in eliminating environmental sources of the virus to control the COVID-19 pandemic.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that is the causative agent of COVID-19 which first emerged in late 2019 [1]. Countries are working to control transmission of SARS-CoV-2 with the ultimate goal of production and large-scale manufacture of an effective vaccine [2-4]. However, until an effective vaccine is found, control of the virus is limited to implementing measures such as contact tracing, quarantine, enforcing strict social distancing, advising frequent hand hygiene and infection control measures in hospital environments [5]. During the 2002 outbreak of SARS-CoV-1, and the 2012 Middle East respiratory syndrome-related (MERS)-CoV outbreak, virus stability facilitated transmission events [6]. Similarly, research has shown that SARS-CoV-2 can remain viable on surfaces, notably plastic and stainless steel for up to 72 hours post inoculation, and

36 in aerosols for at least 3 hours, meaning effective disinfectants can prevent indirect contact
37 transmission [7]. Virusend (TX-10) has been developed to work as a highly effective
38 disinfectant that rapidly inactivates infectious enveloped viruses. As communities begin to
39 reopen and people return to the workplace, effective and quick disinfection of communal
40 areas is paramount to maintaining control of COVID-19. Here we present the evidence that
41 Virusend TX-10 can reduce SAR-CoV-2 virus within one minute both in solution and on
42 surfaces.
43
44 **Methods and Materials**

45 *Cell culture and viruses*

46 Vero E6 cells (C1008: African green monkey kidney cells), obtained from Public Health
47 England, were maintained in Dulbecco's minimal essential medium (DMEM) containing 10%
48 foetal bovine serum (FBS) and 0.05 mg/ml gentamicin. Cells were kept at 37°C with 5% CO_2.
49 Passage 4 or 5 of SARS-CoV-2 isolate (REMRQ0001/Human/2020/Liverpool) from a clinical
50 sample was used to assess inactivation of TX-10. On the fourth and fifth passages the virus
51 was cultured in Vero E6 cells maintained in DMEM with 4% FBS and 0.05mg/mL gentamicin
52 at 37°C and 5% CO_2 as previously described [8]. The fifth passage of the virus was harvested
53 48 hours after inoculation and concentrated by passage through a centrifugal column (Amicon
54 Ultra-15 100kDa MWCO). Virus was used immediately after concentrating.
55
56 *Virus Inactivation*

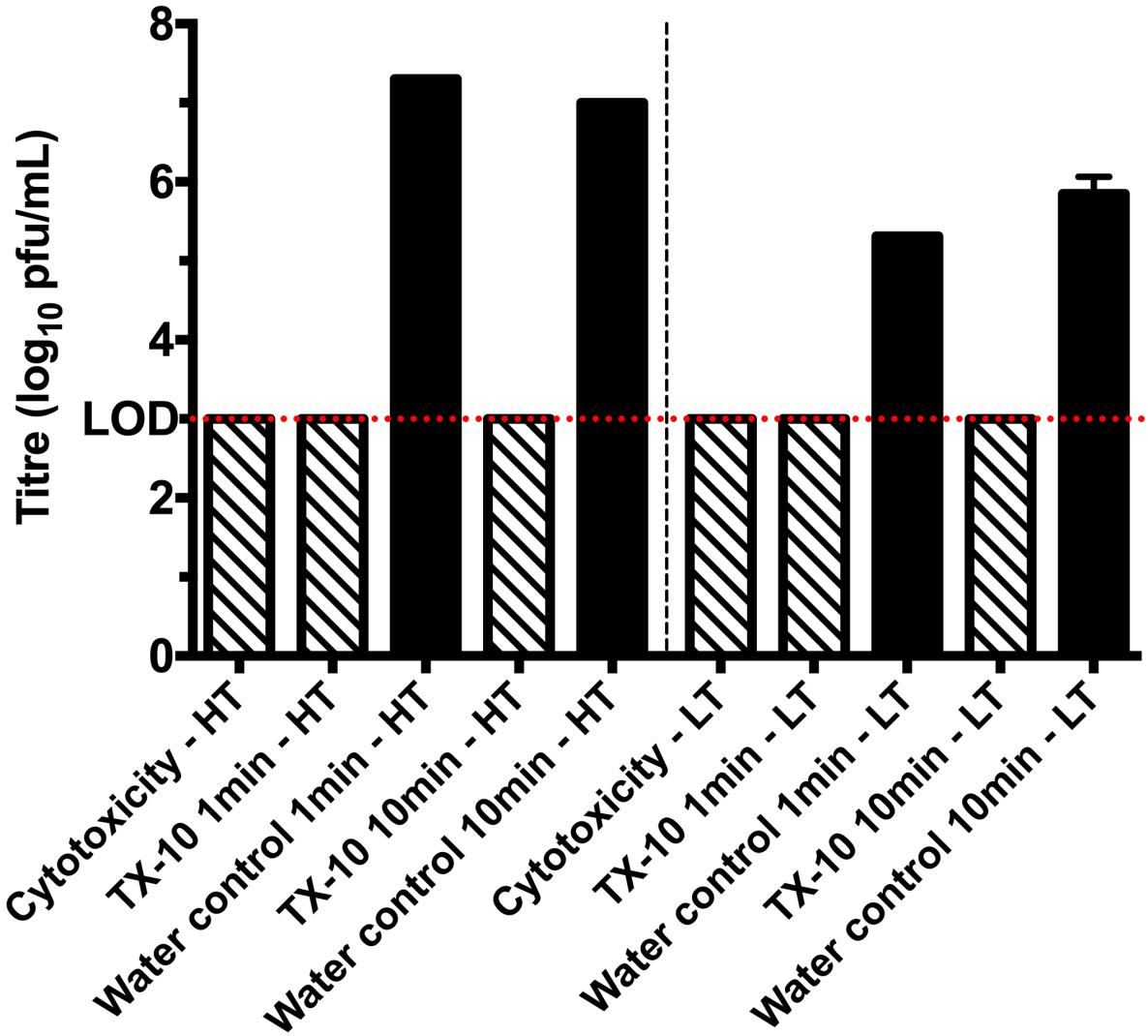
57 Inactivation on surfaces were preformed using either $9.8\log_{10}$ or $7.9\log_{10}$ PFU/mL of SARS-
58 CoV-2. Surface inactivation was carried out by inoculating stainless-steel discs with 50µL of
59 virus and allowed to air dry at room temperature for 1 hour. Dried inoculum was incubated
60 with 100µl of Virusend (TX-10; Pritchard Spray Technologies, Colchester, UK) or autoclaved

61 water for either 30 seconds or 9.5 minutes, after which 900µL of DMEM containing 2% FBS
62 and 0.05 mg/mL gentamicin was added and mixed until dried inoculum was dissolved. The
63 sample was then transferred into a dilution series for virus quantification at exactly 1 minute
64 or 10 minutes after addition of TX-10 to the dried inoculum. Solution inactivation assays used
65 either 8.4$\log_{10}$ or 7.9$\log_{10}$ PFU/mL and were carried out by incubating 25µL of inoculum with
66 100µL of TX-10 or autoclaved water for either 1 minute or 10 minutes. After incubation 10mL
67 of DMEM was added and transferred to a dilution series within 30 seconds of DMEM being
68 added. All experiments were performed in duplicate.
69
70 *Cytotoxicity Assay*
71 Cytotoxicity for surface inactivation was determined by inoculating stainless-steel discs with
72 50µL of DMEM containing 2% FBS and 0.05 mg/mL gentamicin and allowed to air dry at room
73 temperature for 1 hour. Dried inoculum was incubated with 100µl of TX-10 or autoclaved
74 water for 5 minutes, after which 900µL of DMEM containing 2% FBS and 0.05 mg/mL
75 gentamicin was added and mixed until dried inoculum was dissolved. The sample was then
76 transferred into a dilution series and a standard plaque assay performed. Cytotoxicity for
77 solution assays were performed by incubating 25 µL of DMEM containing 2% FBS and 0.05
78 mg/mL gentamicin with 100 µL of TX-10 for 5 minutes, after which 10mL of DMEM was added
79 and sample transferred to a dilution series for standard plaque assays. The cytotoxicity assays
80 were performed in duplicate.
81
82 *Suppression Assay*
83 Suppression for solution inactivation was assayed by adding 25µL of inoculum to 100µL of TX-
84 10 in 10mL of DMEM and incubated for 30 seconds. After 30 seconds, the sample was

85 transferred into a dilution series and a standard plaque assay preformed. The suppression
86 assay was performed in duplicate.
87
88 *Virus Quantification and Viability*
89 Samples from each condition were serial diluted 10-fold for quantification by standard plaque
90 assay using Vero E6 cells. Cells were incubated for 72 hours at 37°C and 5% CO_2, then fixed
91 with 10% formalin and stained with 0.05% crystal violet solution. Plaques were counted to
92 calculate virus titre. All samples were performed in technical duplicates.
93
94 **Results**
95 For inactivation assays, Virusend TX-10 was directly placed on SARS-CoV-2 inoculum, for an
96 incubation period of either 1 minute or 10 minutes. On the hard surface, contact time of 1
97 minute with Virusend TX-10 reduced SARS-CoV-2 titres to below the limit of detection for
98 both high and low titre inoculum (Fig 1). A titre of 7.3$\log_{10}$ PFU/mL was recovered from the
99 high titre, hard surface control samples. Similarly, incubation with Virusend TX-10 for 10
100 minutes reduced the virus titre to below the limit of detection, compared with 7.0$\log_{10}$
101 PFU/mL recovered from the high titre control. With a low titre inoculum, Virusend TX-10 also
102 reduced SARS-CoV-2 titres to below the limit of detection after contact times of 1 and 10
103 minutes on hard surfaces. Titres of 5.3$\log_{10}$ PFU/mL and 5.9$\log_{10}$ PFU/mL were recovered from
104 the 1- and 10-minute control samples, respectively. Cytotoxicity assays with Virusend TX-10
105 in the absence of virus were used to determine the limit of detection, the point at which Vero
106 E6 cell death is due to the cytotoxicity of Virusend TX-10, and not virus. Cytopathic effect was
107 observed to 3.0$\log_{10}$ PFU/mL (Fig. 1). Both inactivation and cytotoxicity assays confirm a

108 reduction of at least 4.0$\log_{10}$ PFU/mL of infectious SARS-CoV-2 with high titre inoculum and a

109 reduction of at least 2.3$\log_{10}$ PFU/mL with low titre inoculum (Fig 1).



110
111 **Fig 1.** Virusend TX-10 reduces viral titre on hard surfaces by at least 4.0$\log_{10}$ PFU/mL with
112 high titre (HT) viral inoculum after contact times of 1 minute and 10 minutes. When low titre
113 (LT) inoculum was used, TX-10 reduces virus titre by at least a 2.31$\log_{10}$ PFU/mL at both 1
114 minute and 10-minute contact time. Diagonal pattern represents cytopathic effect caused by
115 TX-10 and solid black represents the titre of infectious virus following each treatment. Limit
116 of detection (LOD) (3.0$\log_{10}$ PFU/mL) is shown across the graph with a dotted red line.
117

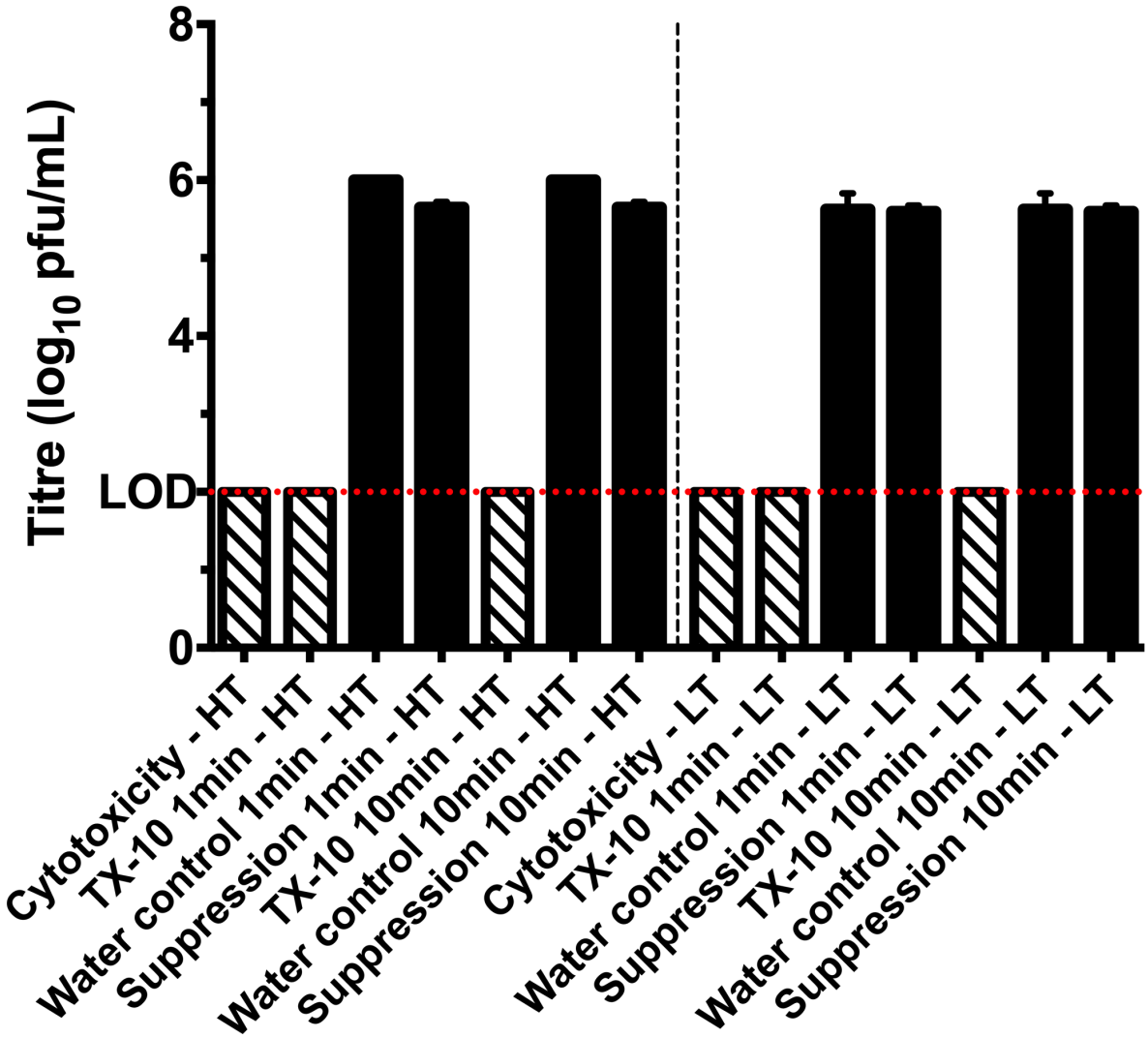
118 For inactivation assays in solution, Virusend TX-10 was placed directly into solution with SARS-

119 CoV-2 for either 1 or 10 minutes. An incubation period of 1 minute with Virusend TX-10

120 reduced the high titre inoculum from 6.00$\log_{10}$ PFU/mL, in the water control, to below the

121 limit of detection (Fig 2A). A 10-minute incubation with Virusend TX-10 also reduced viral titre

122 from 6.0log$_{10}$ PFU/mL to below the limit of detection (Fig 2B). With the low titre inoculum,
123 the addition of Virusend TX-10 reduced SARS-CoV-2 to below the limit of detection at both 1
124 minute and 10 minute incubation times (Fig 2). Titres of 5.6log$_{10}$ PFU/mL were recovered from
125 control samples at 1 minute and 10 minutes. A suppression assay for solution inactivation
126 assays was used to demonstrate that dilution with 10mL of DMEM suppressed Virusend TX-
127 10 inactivation of SARS-CoV-2 upon the completion of the assay. The addition of Virusend TX-
128 10 to virus inoculum in 10mL of DMEM recovered a virus titre of 5.7log$_{10}$ PFU/mL with high
129 titre inoculum and 5.6log$_{10}$ PFU/mL with low titre inoculum. Cytotoxicity assays for solution
130 inactivation assays showed the limit of detection for these assays was 2.0log$_{10}$ PFU/mL.



131

132 **Fig 2.** Virusend TX-10 reduces viral titre in solution by at least 4.0$\log_{10}$ PFU/mL when
133 incubated with high titre (HT) virus inoculum for 1 minute and 10 minutes. When low titre
134 (LT) inoculum was used, both incubation periods reduced the titre by at least 3.6$\log_{10}$ PFU/mL,
135 to below the limit of detection. Diagonal pattern represents cytopathic effect caused by TX-
136 10 and solid black represents the titre of infectious virus following each treatment. Limit of
137 detection (LOD) (2.0$\log_{10}$ PFU/mL) is shown across the graph with a dotted red line.
138
139
140 **Discussion**
141
142 SARS-CoV-2 can remain viable on surfaces, notably plastic and stainless steel for up to 72
143 hours post inoculation, and in aerosols for at least 3 hours [7]. In solutions, such as respiratory
144 droplets, SARS-CoV-2 may remain viable for up to 14 days at 4°C, 7 days at room temperature,
145 and for 1 to 2 days at 37°C [9]. Therefore, contaminated surfaces and solutions are a reservoir
146 for transmission through fomites, meaning effective hygiene and environmental
147 decontamination is crucial in helping to prevent the spread of COVID-19 [10, 11]. Disinfectant
148 solutions of 75% ethanol and 10% sodium hypochlorite are able to reduce SARS-CoV-2 titre
149 by at least 2.0$\log_{10}$ PFU/mL and 3.25$\log_{10}$ PFU/mL, respectively, within 5 minutes [9].
150 However, the WHO has recommended diluting household bleach 1:100 to reduce irritation to
151 the user and contact times of 10 to 60 minutes to disinfect surfaces and when immersing
152 items [12]. Rapid household disinfectants could reduce transmission in private residence and
153 public spaces, such as offices. Here we have shown that Virusend TX-10 is able to reduce
154 SARS-CoV-2 virus titre by at least 4.0$\log_{10}$ PFU/mL in 1 minute of contact time making it and
155 effective disinfectant for households and public spaces.
156
157 An initial obstruction to the work presented here, was the need for a high virus titre to show
158 a 4.0$\log_{10}$ PFU/mL reduction due to the cytotoxicity of Virusend TX-10 to Vero E6 cells. The
159 limit of detection indicated the point at which cytopathic effect in Vero E6 cells is caused by
160 Virusend TX-10 and not the virus. Therefore, to achieve a 4.0$\log_{10}$ PFU/mL reduction, SARS-

161 CoV-2 had to be concentrated after harvesting to give stock titres of 8.4$\log_{10}$ and 9.8$\log_{10}$
162 PFU/mL. When a lower stock virus titre of 7.9$\log_{10}$ PFU/mL was used, a 4.0$\log_{10}$ PFU/mL
163 reduction could not be demonstrated and would not meet the strict requirements of
164 European Standard testing. However, these assays still showed a similar trend of inactivation.
165 The use of higher viral titre in these assays indicates the effectiveness of Virusend TX-10,
166 which may be necessary to inactivate SARS-CoV-2 in environments that are contaminated
167 [13].
168
169 Disinfectants tested for use against other members of *Coronaviridae* have used surrogates,
170 such as murine hepatitis virus, a lower biosafety level pathogen that can be grown to high
171 titres and has structural and genetic similarities to SARS-CoV, to be able to carry out the assays
172 more easily [14]. Surrogates are chosen to mimic the inactivation of the target virus, but the
173 use of surrogates should be limited, and the target pathogen should be used when possible
174 [15]. Here we have been able to test Virusend TX-10 against SARS-CoV-2, the causative agent
175 of the current pandemic, therefore the ability of Virusend TX-10 to significantly reduce the
176 viral titre of the relevant virus.
177
178 Virusend TX-10 is a detergent-based disinfectant. Other detergents, NP-40 and Triton X-100,
179 have been shown to completely inactivate SARS-CoV-2 at a concentration of 0.5% [8]. Both
180 Triton X-100 and NP-40 have also been shown to inactivated the small enveloped hepatitis C
181 virus to below detectable levels, and Triton X-100 inactivates HIV-1 virus completely within 1
182 minute [16]. Like Virusend TX-10, the ability of detergents to inactivate harmful viruses mean
183 they are important for disinfecting contaminated surfaces and solutions. The development of

184 Virusend TX-10, and showing it is highly efficient at inactivating the circulating strain of SARS-
185 CoV-2 specifically, is important to minimise community transmission of SARS-CoV-2.
186
187 Current advice focuses on increasing public engagement in essential control measures, such
188 as high levels of hygiene in the home [17]. Virusend TX-10 can reduce the strain of demand
189 on current hygiene product resources, to be used within private residences, communal public
190 areas such as offices and hospital environments [18-20]. It can reduce viral titres on surfaces
191 and in solution by at least 4.0$\log_{10}$ PFU/mL within 1 minute of contact making it highly suitable
192 for rapid disinfection of private households and public spaces such as hospitals and offices.
193 The development of disinfectants such as Virusend TX-10 and others is important as we
194 continue efforts to reduce transmission of SARS-CoV-2.
195
196 **Acknowledgements**
197 This work was supported by the Ministry of Defence. GLH was supported by the BBSRC
198 (BB/T001240/1 and V011278/1), a Royal Society Wolfson Fellowship (RSWF\R1\180013), the
199 NIH (R21AI138074), URKI (20197), and the NIHR (NIHR2000907). GLH is affiliated to the
200 National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in
201 Emerging and Zoonotic Infections at University of Liverpool in partnership with Public Health
202 England (PHE), in collaboration with Liverpool School of Tropical Medicine and the University
203 of Oxford. GLH is based at LSTM. The views expressed are those of the author(s) and not
204 necessarily those of the NHS, the NIHR, the Department of Health or Public Health England.
205 EIP was supported by the Liverpool School of Tropical Medicine Director's Catalyst Fund
206 award.
207

208 **References**

209

210 1. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus
211 associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.
212 Epub 2020/02/06. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508;
213 PubMed Central PMCID: PMCPMC7094943.

214 2. Sharpe HR, Gilbride C, Allen E, Belij-Rammerstorfer S, Bissett C, Ewer K, et
215 al. The early landscape of coronavirus disease 2019 vaccine development in the UK
216 and rest of the world. Immunology. 2020;160(3):223-32. Epub 2020/05/28. doi:
217 10.1111/imm.13222. PubMed PMID: 32460358; PubMed Central PMCID:
218 PMCPMC7283842.

219 3. Yamey G, Schaferhoff M, Hatchett R, Pate M, Zhao F, McDade KK. Ensuring
220 global access to COVID-19 vaccines. Lancet. 2020;395(10234):1405-6. Epub
221 2020/04/04. doi: 10.1016/S0140-6736(20)30763-7. PubMed PMID: 32243778;
222 PubMed Central PMCID: PMCPMC7271264.

223 4. Thanh Le T, Andreadakis Z, Kumar A, Gomez Roman R, Tollefsen S, Saville
224 M, et al. The COVID-19 vaccine development landscape. Nat Rev Drug Discov.
225 2020;19(5):305-6. Epub 2020/04/11. doi: 10.1038/d41573-020-00073-5. PubMed
226 PMID: 32273591.

227 5. Cheng VC, Wong SC, Chuang VW, So SY, Chen JH, Sridhar S, et al. The role
228 of community-wide wearing of face mask for control of coronavirus disease 2019
229 (COVID-19) epidemic due to SARS-CoV-2. J Infect. 2020;81(1):107-14. Epub
230 2020/04/27. doi: 10.1016/j.jinf.2020.04.024. PubMed PMID: 32335167; PubMed
231 Central PMCID: PMCPMC7177146.

232 6. Xiao S, Li Y, Wong TW, Hui DSC. Role of fomites in SARS transmission during
233 the largest hospital outbreak in Hong Kong. PLoS One. 2017;12(7):e0181558. Epub
234 2017/07/21. doi: 10.1371/journal.pone.0181558. PubMed PMID: 28727803; PubMed
235 Central PMCID: PMCPMC5519164.
236 7. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A,
237 Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with
238 SARS-CoV-1. N Engl J Med. 2020;382(16):1564-7. Epub 2020/03/18. doi:
239 10.1056/NEJMc2004973. PubMed PMID: 32182409; PubMed Cen
240 tral PMCID: PMCPMC7121658.
241 8. Patterson EI, Prince T, Anderson ER, Casas-Sanchez A, Smith SL, Cansado-
242 Utrilla C, et al. Methods of Inactivation of SARS-CoV-2 for Downstream Biological
243 Assays. J Infect Dis. 2020;222(9):1462-7. Epub 2020/08/17. doi:
244 10.1093/infdis/jiaa507. PubMed PMID: 32798217; PubMed Central PMCID:
245 PMCPMC7529010.
246 9. Chan KH, Sridhar S, Zhang RR, Chu H, Fung AY, Chan G, et al. Factors
247 affecting stability and infectivity of SARS-CoV-2. J Hosp Infect. 2020;106(2):226-31.
248 Epub 2020/07/12. doi: 10.1016/j.jhin.2020.07.009. PubMed PMID: 32652214;
249 PubMed Central PMCID: PMCPMC7343644.
250 10. Hellewell J, Abbott S, Gimma A, Bosse NI, Jarvis CI, Russell TW, et al.
251 Feasibility of controlling COVID-19 outbreaks by isolation of cases and contacts.
252 Lancet Glob Health. 2020;8(4):e488-e96. Epub 2020/03/03. doi: 10.1016/S2214-
253 109X(20)30074-7. PubMed PMID: 32119825; PubMed Central PMCID:
254 PMCPMC7097845.
255 11. Ogbunugafor CB, Miller-Dickson MD, Meszaros VA, Gomez LM, Murillo AL,
256 Scarpino SV. The intensity of COVID-19 outbreaks is modulated by SARS-CoV-2 free-

257 living survival and environmental transmission. medRxiv. 2020. Epub 2020/06/09. doi:
258 10.1101/2020.05.04.20090092. PubMed PMID: 32511513; PubMed Central PMCID:
259 PMCPMC7273281.
260 12. Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute
261 Respiratory Infections in Health Care. WHO Guidelines Approved by the Guidelines
262 Review Committee. Geneva2014.
263 13. Razzini K, Castrica M, Menchetti L, Maggi L, Negroni L, Orfeo NV, et al. SARS-
264 CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in
265 Milan, Italy. Sci Total Environ. 2020;742:140540. Epub 2020/07/04. doi:
266 10.1016/j.scitotenv.2020.140540. PubMed PMID: 32619843; PubMed Central
267 PMCID: PMCPMC7319646.
268 14. Coley SE, Lavi E, Sawicki SG, Fu L, Schelle B, Karl N, et al. Recombinant
269 mouse hepatitis virus strain A59 from cloned, full-length cDNA replicates to high titers
270 in vitro and is fully pathogenic in vivo. J Virol. 2005;79(5):3097-106. Epub 2005/02/15.
271 doi: 10.1128/JVI.79.5.3097-3106.2005. PubMed PMID: 15709029; PubMed Central
272 PMCID: PMCPMC548458.
273 15. Richards GP. Critical review of norovirus surrogates in food safety research:
274 rationale for considering volunteer studies. Food Environ Virol. 2012;4(1):6-13. Epub
275 2012/03/13. doi: 10.1007/s12560-011-9072-7. PubMed PMID: 22408689; PubMed
276 Central PMCID: PMCPMC3284674.
277 16. Song H, Li J, Shi S, Yan L, Zhuang H, Li K. Thermal stability and inactivation of
278 hepatitis C virus grown in cell culture. Virol J. 2010;7:40. Epub 2010/02/20. doi:
279 10.1186/1743-422X-7-40. PubMed PMID: 20167059; PubMed Central PMCID:
280 PMCPMC2834657.
281 17. Sciences TAoM. Preparing for a challenging winter 2020/21. 2020.

282 18. Dear K, Grayson L, Nixon R. Potential methanol toxicity and the importance of
283 using a standardised alcohol-based hand rub formulation in the era of COVID-19.
284 Antimicrob Resist Infect Control. 2020;9(1):129. Epub 2020/08/11. doi:
285 10.1186/s13756-020-00788-5. PubMed PMID: 32771064; PubMed Central PMCID:
286 PMCPMC7414286.
287 19. Klemes JJ, Fan YV, Jiang P. The energy and environmental footprints of
288 COVID-19 fighting measures - PPE, disinfection, supply chains. Energy (Oxf).
289 2020;211:118701. Epub 2020/09/02. doi: 10.1016/j.energy.2020.118701. PubMed
290 PMID: 32868962; PubMed Central PMCID: PMCPMC7450254.
291 20. Dicken RDG, T.; Perks, S. Overcoming the Regulatory Hurdles for the
292 Production of Hand Sanitizer for Public Health Protection: The UK and US Academic
293 Perspective. ACS Chemical Health and Safety. 2020;27:209-13. doi:
294 10.1021/acs.chas.0c00065.
295