

1       **Comparable environmental stability and disinfection profiles of the currently**  
2       **circulating SARS-CoV-2 variants of concern B.1.1.7 and B.1.351**

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29

30 **Abstract**

31 The emergence of novel SARS-CoV-2 B.1.1.7 and B.1.351 variants of concern with  
32 increased transmission dynamics has raised questions regarding stability and  
33 disinfection of these viruses. In this study, we analyzed surface stability and  
34 disinfection of the currently circulating SARS-CoV-2 variants B.1.1.7 and B.1.351  
35 compared to the wildtype. Treatment with heat, soap and ethanol revealed similar  
36 inactivation profiles indicative of a comparable susceptibility towards disinfection.  
37 Furthermore, we observed comparable surface stability on steel, silver, copper and  
38 face masks. Overall, our data support the application of currently recommended  
39 hygiene concepts to minimize the risk of B.1.1.7 and B.1.351 transmission.

40

41 **Background**

42 Since the outbreak of *Severe Acute respiratory Syndrome Coronavirus-2* (SARS-CoV-  
43 2) at the end of 2019, > 120 million cases and > 2.8 million death (March 31<sup>st</sup> 2021)  
44 have been reported [1]. Viral evolution includes the natural emergence of viral variants,  
45 which can encode for a variety of mutations in their genome compared to the parental  
46 wildtype virus. Mutations which confer either enhanced fitness, higher pathogenicity,  
47 better transmissibility or immune escape are of special concern as they could  
48 significantly influence transmission dynamics with devastating consequences. In-  
49 dependent lineages of SARS-CoV-2 have recently been reported: UK, B.1.1.7; South  
50 Africa, B.1.351; and Brazil, P.1 [2]. Importantly, these variants of concern (VOC)  
51 display higher reproduction numbers than preexisting variants and consequently  
52 increase incidences in various countries. Moreover, VOCs have been associated with  
53 more severe course of infection and/or potential immune escape due to multiple  
54 changes in the immunodominant spike protein [3–5]. Since the global access to  
55 COVID-19 vaccines is still limited, diligent attention on transmission-based  
56 precautions is essential to limit VOC spread. However, given the rapid spread and  
57 increased transmission dynamics of the emerging variants, concerns regarding the  
58 effectiveness of current hygiene measures and inactivation strategies have been  
59 raised. Here we compared the stability of three SARS-CoV-2 strains, the preexisting  
60 B1.1.70 variant (herein referred as WT virus) and the currently emerging B.1.1.7 and  
61 B.1.351 variants on different surfaces and their sensitivity to heat, soap and ethanol.

63 **Methods**

64 *Viral isolates and Cell culture*

65 For SARS-CoV-2 virus suspension preparation, Vero E6 cells (kindly provided by C.  
66 Drosten and M. Müller) were seeded at  $2 \times 10^6$  cells in a 75 cm<sup>2</sup> flask in Dulbecco's  
67 modified Eagle's medium (DMEM, supplemented with 10 % (v/v) fetal calf serum  
68 (FCS), 1 % (v/v) non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL  
69 streptomycin and 2 mM L-Glutamine). After 24 h the cells were inoculated with 100 µL  
70 of either wild type virus hCoV-19/Germany/BY-Bochum-1/2020 (GISAID accession ID:  
71 EPI\_ISL\_1118929), VOC B.1.1.7\_RKI-0026\_B.1.1.7 (GISAID accession ID:  
72 EPI\_ISL\_751799) or the VOC B.1351 RKI-0029\_B.1.351 (GISAID accession ID:  
73 EPI\_ISL\_803957). Spike domains of strains were checked for lineage features prior  
74 to assays in the context of routine diagnostics (primer kindly provided by René  
75 Scholtysik, University Hospital Essen; details about sequences and cycling conditions  
76 available upon request). Three days post infection and upon visible cytopathic effects  
77 virus suspension was harvested by collecting the supernatant and subsequent  
78 centrifugation for 5 min at 1,500 rpm to remove any cell debris. The virus suspensions  
79 were aliquoted and stored at -80 °C until further usage.

80 *Carrier assay*

81 To analyze viral stability on different surfaces we performed time kinetics and studied  
82 viral stability over 48 h. Therefore, stainless steel disk, disks sputtered with copper or  
83 silver, the inner layer of surgical masks and Filtering Face Piece 2 (FFP2) masks were  
84 inoculated with  $5 \times 10 \mu\text{L}$  of test virus suspension. The test suspension contained 9-  
85 parts virus and 1-part interfering substance (bovine serum albumin [BSA], 0.3g/L in  
86 phosphate buffered saline [PBS] according to EN 5.2.2.8) and was adjusted to  $5 \times 10^6$

87 TCID<sub>50</sub>/mL. Immediately, 10 min, 1 h, 24 h and 48 h after virus inoculation on the  
88 different surfaces they were placed aseptically in a 2 ml DMEM (without FCS)  
89 harboring container and vortexed for 60 s. To determine the amount of recovered  
90 infectious virus from the test specimen an end-point-dilution assay was performed on  
91 Vero E6 cells to calculate the remaining TCID<sub>50</sub> according to Spearman and Kärber  
92 [6, 7].

93 *Quantitative suspension assay*

94 To test susceptibility to disinfection, viruses were exposed to 20, 30, 40, 60 and 80 %  
95 (v/v) ethanol for 30 s or to hand soap (Lifosan® soft, B. Braun Medical AG, diluted  
96 1:49 in water) for 30 s, 1 min, 5 min and 10 min. Therefore, 8-parts ethanol or hand  
97 soap were mixed with 1-part interfering substance (BSA, 0.3g/L in PBS according to  
98 EN 5.2.2.8) and 1-part virus adjusted to 5×10<sup>6</sup> TCID<sub>50</sub>/mL. The suspensions were  
99 incubated for the indicated time periods and residual viral infectivity was determined  
100 by performing an end point dilution assay on Vero E6 cells.

101 *Heat inactivation*

102 To access susceptibility towards heat virus suspension were incubated for 1 min, 5  
103 min, 10 min and 30 min at 56 °C. Thus, 9 parts virus adjusted to 5×10<sup>6</sup> TCID<sub>50</sub>/mL  
104 were mixed with 1 part interfering substance (BSA, 0.3g/L in PBS according to EN  
105 5.2.2.8) and incubated for the indicated time periods. Reduction of viral titers were  
106 examined by end point dilution assay to calculate TCID<sub>50</sub> values.

107

108 **Results**

109 In order to address if the newly emerged VOC B.1.1.7 and B.1.351 were equally  
110 susceptible towards different inactivation strategies as the wild type virus we  
111 compared viral inactivation upon usage of ethanol, a common ingredient of several  
112 disinfectants and recommended by the World Health Organization (WHO) in resource  
113 limited countries [8]. Viruses were exposed towards increasing concentrations of  
114 ethanol for 30 s and residual viral infectivity was determined by endpoint titration. In  
115 accordance to previous results, all three viral variants could be efficiently inactivated  
116 upon treatment with at least 30 % (v/v) ethanol for 30 s, confirming equal susceptibility  
117 towards disinfection (**Figure 1**). Since disinfection procedures are mainly  
118 recommended in clinical setups, we next addressed the virucidal activity of  
119 conventional hand soap. SARS-CoV-2 variants were inoculated with a 1:49 dilution of  
120 commercially available hand soap and viral infectivity determined after different time  
121 points. All viral variants were effectively inactivated after exposure towards soap within  
122 1 - 5 minutes, supporting current hygiene measures (**Figure 1**). Next, we addressed  
123 susceptibility of the three strains towards heat (56°C) and observed a decrease in viral  
124 titers towards background levels within 30 min. Importantly, inactivation kinetics were  
125 comparable between all viral variants (**Figure 1**). Although SARS-CoV-2 is mainly  
126 transmitted through respiratory droplets and aerosols exhaled from infected  
127 individuals transmission via fomites cannot be excluded. Viral stability was examined  
128 on representative materials surfaces: silver, copper and stainless-steel discs for up to  
129 48 h, using an initial virus concentration of  $9.2 \times 10^6$  TCID<sub>50</sub>/mL. Importantly, all  
130 variants remained infectious on the different surfaces for 48 h and compared to the  
131 wildtype virus no differences in the relative infectivity were observed (**Figure 2A**). In  
132 order to mimic a potential contamination of on protective masks by infected individuals,

133 we contaminated the inside of either a surgical mask or a FFP2 mask and analyzed  
134 viral stability for all variants. Again, comparable residual titers of all VOCs were  
135 observed over time (**Figure 2B**). In conclusion, the currently circulating VOC did not  
136 exhibit enhanced surface stability or differences in disinfection profiles indicating that  
137 current hygiene measures are sufficient and appropriate.

138

139 **Discussion**

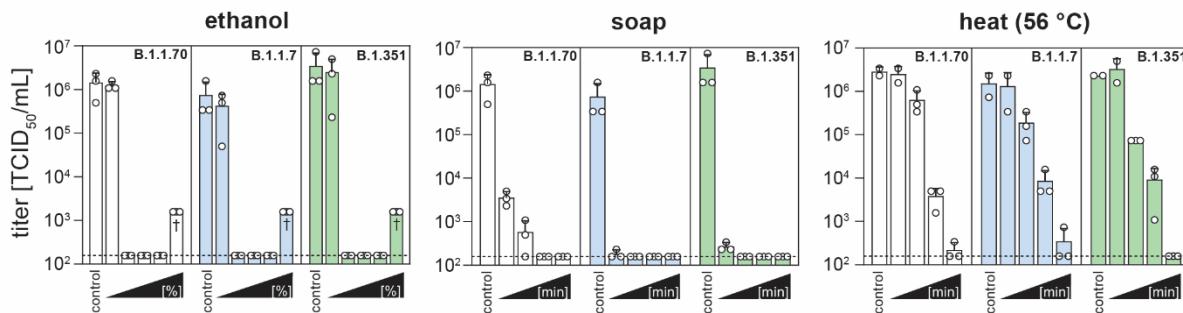
140 The currently circulating VOCs, including B.1.1.7 and B.1.351 have shown a strong  
141 increase in incidences in various countries. In particular, the B.1.1.7 strain has been  
142 suspected to display a 43–90% higher reproduction number compared to preexisting  
143 variants [3, 9]. However, the exact mechanisms underlying the increased transmission  
144 rates are still under investigation. Given the challenges during the rollout of COVID-19  
145 vaccines, current prevention measures are based on the “swiss cheese model” [10],  
146 including a combination of different intervention strategies. In most countries, physical  
147 distancing, face covers and hygiene measures are the main strategies to lower virus  
148 spread. Therefore, it is essential to address if current hygiene strategies are sufficient  
149 and appropriate to prevent transmission of newly emerging VOCs. Especially in the  
150 hospital setting, viral disinfection is crucial given the large number of infected patients  
151 with high viral loads in a limited space. Several disinfectants are based on ethanol  
152 which has been shown to efficiently inactivate CoVs within a very short time frame  
153 [11]. In agreement with this, we observed a comparable susceptibility of all viral  
154 variants tested towards a minimum of 30 % ethanol upon 30 s exposure, indicative of  
155 similar disinfection properties. Since disinfections are not recommended for the daily  
156 use, we further examined the virucidal efficiency of common household soap. Soaps  
157 contain a mixture of surfactants, which can act directly antiviral upon insertion into the  
158 lipid envelope thereby leading to the disintegration of the virus within minutes [12, 13].  
159 However, given that common day-to-day practices do normally not include soaping of  
160 hands for several minutes, additional effects can include viral elution from the hand  
161 surface due to the adsorptive properties of soap that results upon hand rubbing and  
162 subsequent washing in successful removal of the viral particles [14]. We observed an  
163 efficient inactivation of all variants within 30 s exposure and upon 5 min all viral variants

164 were completely inactivated. Of note, contact times can differ depending on the ratio  
165 of soap and water. Interestingly, we observed slight differences with a minimal residual  
166 infectivity after 30 s and 1 min for the wildtype in contrast to the tested VOCs. However,  
167 these could be attributed to a variety of factors and do not necessarily reflect changed  
168 biological properties of the viruses. In order to minimize the risk of SARS-CoV-2  
169 transmission while handling and processing of clinical specimens, standard  
170 precautions involve different inactivation procedures to reduce or abolish infectivity.  
171 Heat inactivation protocols are commonly used for a variety of subsequent  
172 applications, therefore, we aimed to address the susceptibility of VOCs towards  
173 treatment with 56 °C for different times. As described before, a 30 min treatment with  
174 56 °C is sufficient to efficiently abolish infectivity, with no differences between the  
175 VOCs. Transmission via contaminated surfaces (fomites) is not considered to be a  
176 main route of infection, nevertheless given the high transmission rates questions  
177 regarding changed environmental stability were being raised. Surface stability for  
178 several days has been described under laboratory conditions for several  
179 coronaviruses [15–17]. Using different surfaces, we did not observe any differences  
180 regarding viral decay kinetics. Importantly, we observed prolonged stability of all  
181 variants on face masks, highlighting the importance of exchanging masks regularly  
182 and the risk of shared masks. Of note, in contrast to other publications [18], we did not  
183 observe an antiviral effect of silver surfaces on SARS-CoV-2. This is in contrast to  
184 copper, for which antiviral properties have been described before and could be  
185 confirmed in this study [19]. In conclusion, our results suggest that current hygiene  
186 measures are appropriate and effective against the currently circulating VOCs.

187

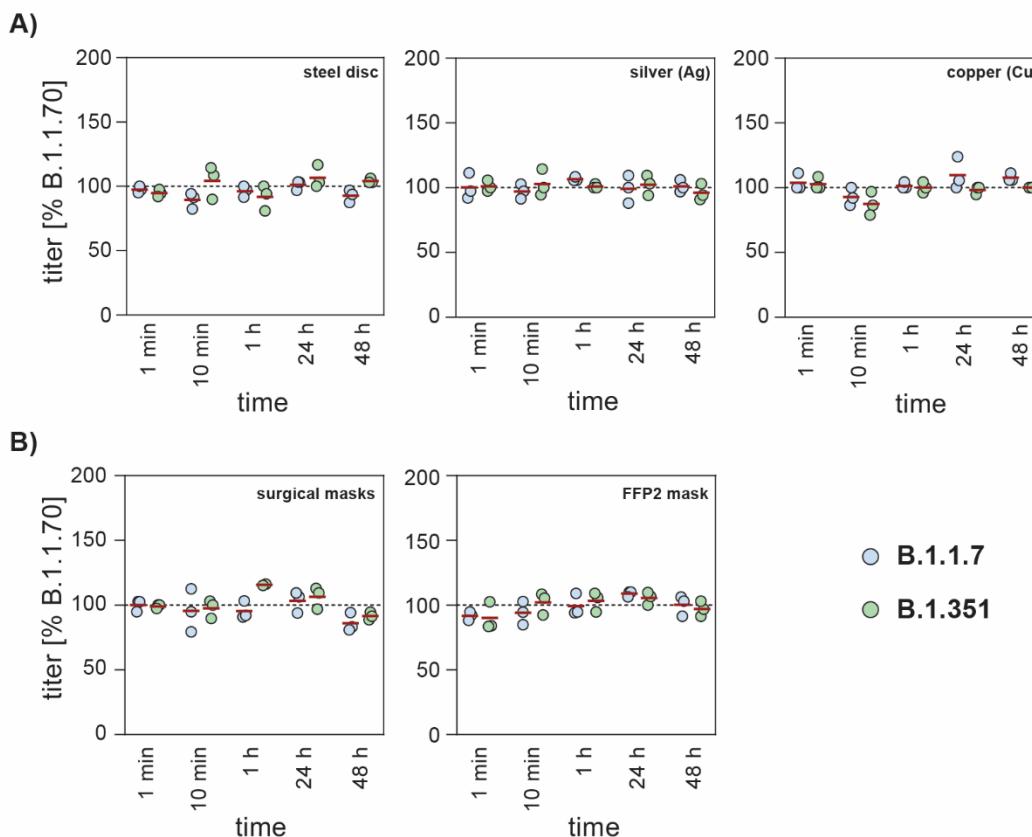
188 **Figure legends**

189 **Figure 1**



190 **Figure 1: Inactivation of SARS-CoV-2 B.1.1.7 and B.1.351 variants compared to**  
191 **B.1.1.70 (wild type).** Residual titer (TCID<sub>50</sub>/mL) of B.1.1.70 (white bars) B.1.1.7 (blue  
192 bars) and B.1.351 (green bars) variants after inactivation via heat (56 °C, left panel)  
193 for 1, 5, 10 and 30 min (left to right), soap (middle panel) for 30 s, 1, 5 and 10 min (left  
194 to right) and ethanol (right panel, 20%, 30%, 40%, 60% and 80%, left to right).  
195 Depicted are the individual replicates as dots and the mean as bars ± SD; dashed line  
196 indicates lower limit of quantification (LLOQ) of the limiting dilution assay. † denotes  
197 elevated LLOQ due to cytotoxicity.

Figure 2



198

199 **Figure 2: Relative stability of SARS-CoV-2 B.1.1.7 and B.1.351 variants to**  
200 **B.1.1.70 (wildtype).** SARS-CoV-2 stock solutions were applied on different surfaces  
201 and recovered after the indicated times and residual titer was assessed via limiting  
202 dilution assay (TCID<sub>50</sub>/mL). Normalized stability of B.1.1.7 (blue dots) and B.1.351  
203 (green dots) variants on A) stainless steel discs and disks sputtered with copper or  
204 silver and B) on the inner layer of surgical masks and Filtering Face Piece 2 (FFP2)  
205 masks relative to wild type (dashed line). Depicted are the individual replicates as dots  
206 and the mean as red lines.

207

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215

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