

# Journal Pre-proof



A realistic transfer method reveals low risk of SARS-CoV-2 transmission via contaminated euro coins and banknotes

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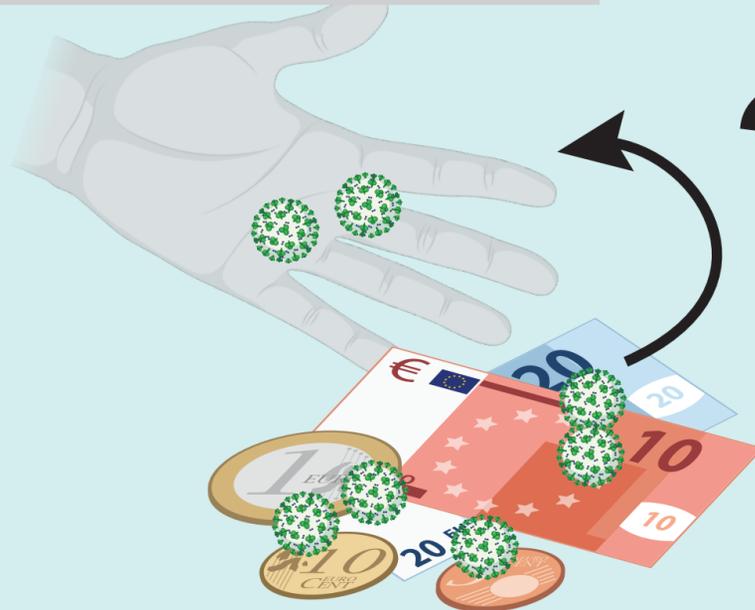
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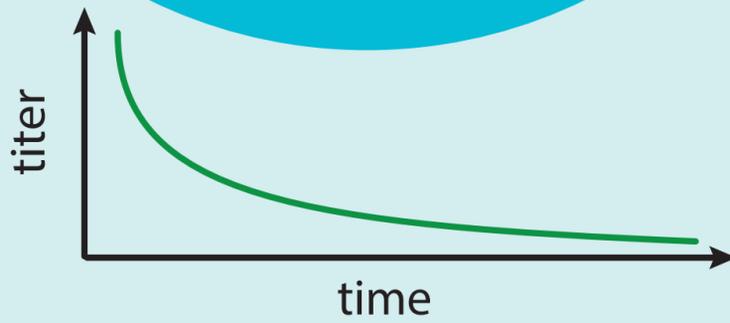
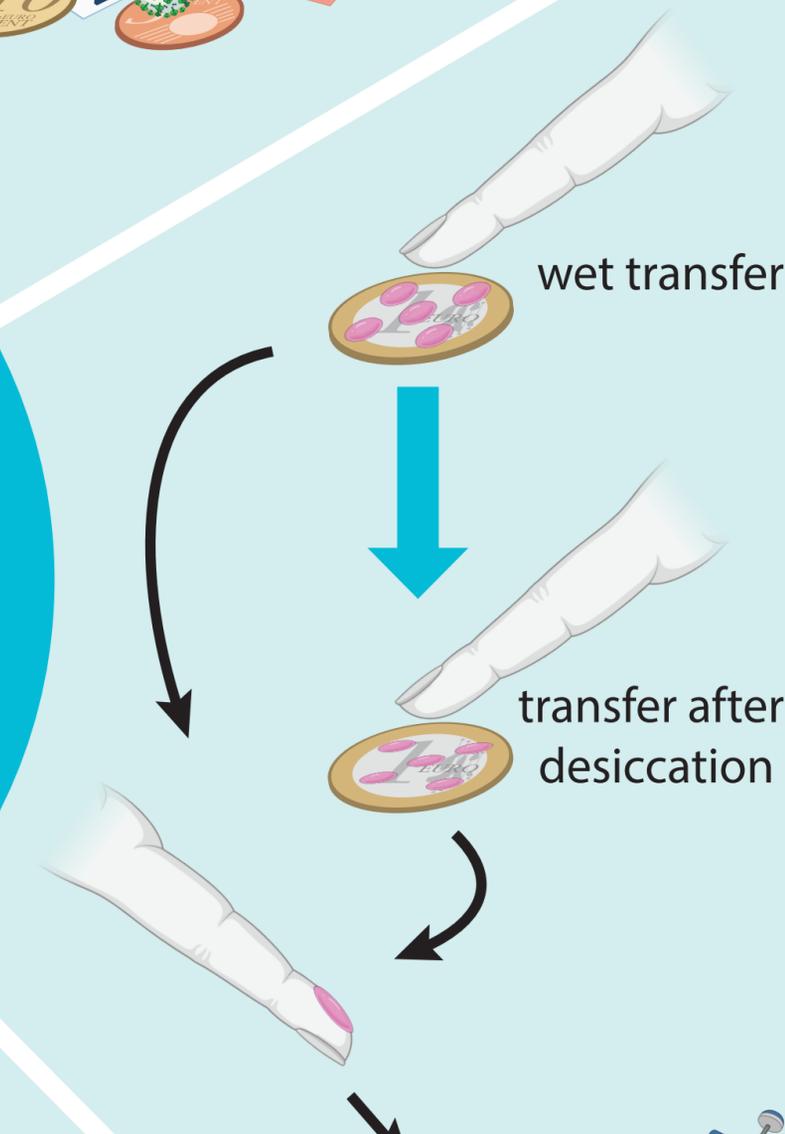
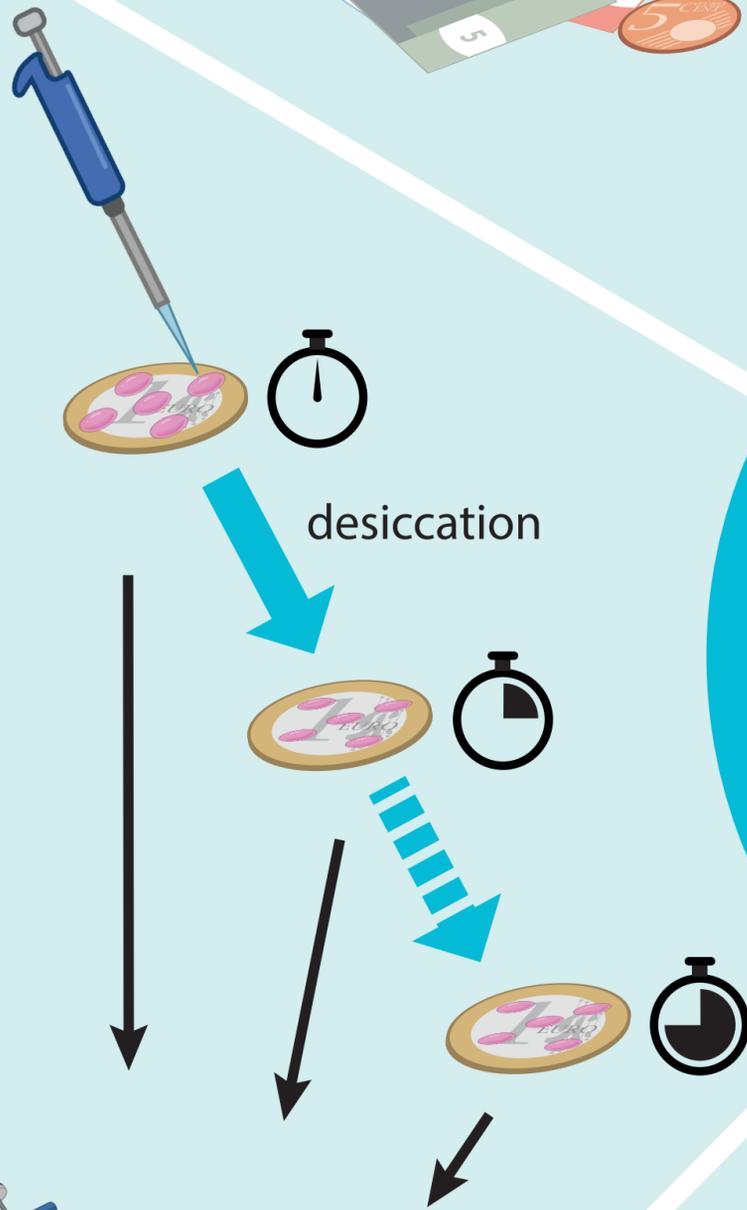
stability

Journal Pre-proof

touch-transfer



# Stability and touch-transfer of SARS-CoV-2



wet transfer | dry transfer



1           **A realistic transfer method reveals low risk of SARS-CoV-2**  
2           **transmission via contaminated euro coins and banknotes**

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45

46 **Abstract**

47 The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic  
48 has created a significant threat to global health. While respiratory aerosols or droplets  
49 are considered as the main route of human-to-human transmission, secretions expelled  
50 by infected individuals can also contaminate surfaces and objects, potentially creating  
51 the risk of fomite-based transmission. Consequently, frequently touched objects such as  
52 paper currency and coins have been suspected as potential transmission vehicle. To  
53 assess the risk of SARS-CoV-2 transmission by banknotes and coins, we examined the  
54 stability of SARS-CoV-2 and bovine coronavirus (BCoV), as surrogate with lower  
55 biosafety restrictions, on these different means of payment and developed a touch  
56 transfer method to examine transfer efficiency from contaminated surfaces to fingertips.  
57 Although we observed prolonged virus stability, our results indicate that transmission of  
58 SARS-CoV-2 via contaminated coins and banknotes is unlikely and requires high viral  
59 loads and a timely order of specific events.

60

61 **Key words:** SARS-CoV-2, stability, coins, banknotes, fingertips

62

**63 Introduction**

64

65 The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic  
66 has created a significant threat to global health. Since effective treatments and access to  
67 vaccines is still limited for the broad population in most countries, diligent attention on  
68 transmission-based precautions is essential to limit viral spread. In particular considering  
69 the emergence of novel SARS-CoV-2 variants displaying increased transmissibility,  
70 more severe disease and significant reduction in neutralization by antibodies can reduce  
71 the effectiveness of treatments or vaccines.(Nicholas G. Davies et al. 2021; Hou et al.  
72 2020). According to current evidence, SARS-CoV-2 is mainly transmitted through  
73 respiratory droplets and aerosols exhaled from infected individuals (Kampf et al. 2020).  
74 Respiratory secretions or droplets expelled by infected individuals can potentially  
75 contaminate surfaces and objects (fomites) and have been shown to persist on inanimate  
76 surfaces for days under controlled laboratory conditions (Kratzel et al. 2020; van  
77 Doremalen et al. 2020). Therefore, a clinically significant risk of SARS-CoV-2  
78 transmission by fomites has been assumed (Ong et al. 2020; Riddell et al. 2020; Chia et  
79 al. 2020). The COVID-19 pandemic intensified the decline in the transactional use of  
80 cash, partly due to reduced consumer spending, but also due to concerns about the risk  
81 of banknotes transmitting the virus. This was observed for both sides, the retailers' as  
82 well as the customers (European Central Bank 2021). Indeed, frequently touched objects  
83 such as banknotes and coins have been suspected to serve as transmission vehicle of  
84 various pathogenic bacteria, parasites, fungi and viruses including SARS-CoV-2  
85 (Angelakis et al. 2014; Pal and Bhadada 2020). However, the conditions presented in  
86 various experimental studies often do not resemble real-life scenarios (e.g. large virus

87 inoculums, small surface area) and thereby potentially exaggerating the risk of  
88 transmission of SARS-CoV-2 by fomites (Mondelli et al. 2020; Goldman 2020).  
89 Although different viruses are readily exchanged between skin and surfaces, the fraction  
90 of virus transferred is dependent on multiple factors including virus species and surface  
91 material (Julian et al. 2010). The efficiency of pathogen transfer from the fomite to hands  
92 is an important parameter to model its potential for transmission and to implement  
93 effective hygiene measures, while avoiding unnecessary measures (Lopez et al. 2013).  
94 However, the transfer of SARS-CoV-2 from surfaces to skin has not been analyzed  
95 systematically. Here, we examined the stability of SARS-CoV-2 and bovine coronavirus  
96 (BCoV) as surrogate on different means of payment. We further implemented a new  
97 protocol to study the touch transfer efficiency between fomites and fingertips.  
98 Importantly, we only observed a transfer between fomites and fingertips using a large  
99 initial virus titer sample ( $10^6$  50% tissue culture infectious dose per milliliter  
100 ( $TCID_{50}/mL$ )) on the tested surfaces, while lower initial virus titer stocks ( $10^4$   
101  $TCID_{50}/mL$ ) were not effectively transferred.

102 Overall, our results point to a low risk of SARS-CoV-2 transmission by coins and  
103 banknotes and the tendency to prefer contactless payment over cash during the pandemic  
104 seems unnecessary.

105

## 106 **Results**

107

### 108 **Stability of BCoV on euro banknotes**

109 To examine the stability of coronaviruses on banknotes, we first used bovine coronavirus  
110 (BCoV), which can be cultivated under lower biosafety levels and has been used as a  
111 surrogate virus for inactivation studies replacing the highly pathogenic MERS-CoV and  
112 SARS-CoV (Siddharta et al. 2017). All euro banknotes are made of pure cotton fiber. To  
113 protect the surface of banknotes with smaller denomination and prolong circulation life,  
114 5 € and 10 € banknotes are coated with a varnish applied after printing (European Central  
115 Bank 2017). To account for the effect of this varnish on surface stability of BCoV over  
116 time, we assessed residual infectivity from pieces of 10 € and 50 € banknotes for 7 h, 24  
117 h and subsequently every 24 – 48 h up to 7 days (Figure 1A). The initial virus  
118 concentration of  $4.3 \times 10^6$  TCID<sub>50</sub>/mL declined to  $1.84 \times 10^4$  TCID<sub>50</sub>/mL on 10 €  
119 banknotes and  $9.25 \times 10^4$  TCID<sub>50</sub>/mL on 50 € banknotes after 7 h desiccation. To  
120 quantitatively compare this early loss of titer on the different surfaces, we employed a  
121 fitted Weibull distribution model to estimate initial decay rates and the modelled time to  
122 (LLOQ) (Figure 1B, Table 1). For both banknotes we observed shorter initial decay (2.75  
123 h on 50€ and 6.45 h on 10€) as compared to the steel disc (49.62 h) (Figure 1B, Table  
124 1). Following the strong initial decay, we were able to detect low amounts of infectious  
125 virus after 120 h (50€) and 168 h (10€) respectively (Figure 1A), which is very much in  
126 line with the observed times in the model of 175.62 h for 50€ and 216.31 h for 10€ notes  
127 (Figure 1B and Table 1). In contrast, on steel discs a more continuous decay was  
128 observed and infectious virus could be recovered up to 120 h (Figure 1A), and 229.73 h  
129 for the fitted model, respectively (Figure 1B, Table 1).

130

131 **Stability of SARS-CoV-2 on euro banknotes and coins**

132 We next examined the surface stability of infectious SARS-CoV-2 on 10 € banknotes,  
133 different coins (1 €, 10 cent, 5 cent) and stainless-steel discs for up to 7 days using an  
134 initial virus concentration of  $1.36 - 2.0 \times 10^6$  TCID<sub>50</sub>/mL (Figure 2). On 10 € banknotes  
135 and 1 € coins, the initial virus concentration declined to  $2.32 \times 10^4$  TCID<sub>50</sub>/mL and  $1.79$   
136  $\times 10^4$  TCID<sub>50</sub>/mL, respectively, after 1.25 h, corresponding to an estimated initial decay  
137 time of 6.07 h and 2.21 h (Figure 2B, Table 1). No infectious virus could be recovered  
138 after 72 h and 48 h (Figure 2A) matching 85.67 h and 28.43 h survival time (Figure 2B,  
139 Table 1). In contrast, on 10 cent and 5 cent coins the initial virus concentration declined  
140 to  $5.96 \times 10^4$  TCID<sub>50</sub>/mL and  $3.86 \times 10^1$  TCID<sub>50</sub>/mL, respectively, within 30 min. Initial  
141 decay rates were calculated as 49.8 min (10 cent) and 12 min (5 cent) (Figure 2B, Table  
142 1). Importantly, from 10 cent coins no infectious virus could be recovered after 6 h, while  
143 for 5 cent coins infectivity was completely lost after 1 h (Figure 2A), as reflected by 2.28  
144 h and 33 min survival time for SARS-CoV-2 on 10 cent and 5 cent coins (Figure 2B,  
145 Table 1). In contrast, on stainless-steel discs, which served as reference material, initial  
146 decay and time to reach background levels were comparable to BCoV with 20.59 h and  
147 158.83 h, respectively (Figure 2B, Table 1). Virus titers declined more evenly until no  
148 infectious virus could be recovered after 120 h (Figure 2A). Likewise, we examined the  
149 surface stability of the SARS-CoV-2 alpha variant on 10 € banknotes, different coins (1  
150 €, 10 cent, 5 cent) and stainless-steel discs for up to 7 days using an initial virus  
151 concentration of  $6.87 \times 10^6$  TCID<sub>50</sub>/mL (Figure S1). On all surfaces, the initial virus  
152 concentration declined to  $\sim 5 - 1 \times 10^4$  TCID<sub>50</sub>/mL after 0.25 h (Figure S1A). However,  
153 on 10 € banknotes, 1 € and 10 cent coins no infectious virus could be recovered after 7

154 h and 24 h, while on 5 cent coins the virus was already inactivated after 2 h. In contrast,  
155 on stainless-steel discs, following the initial decay, infectious virus could be recovered  
156 for up to 7 days. Quantitative estimates of the initial decay rates were comparable to wild  
157 type SARS-CoV-2 (Figure S1B, Table 1). Time to reach LLOQ was decreased on 10 €  
158 banknotes, while on coins slightly longer times were modelled (Table 1). Overall, we  
159 observed comparable inactivation kinetics on the different materials for the SARS-CoV-  
160 2 alpha variant of concern when compared to the wild type virus.

161

### 162 **Development of a touch transfer assay to study virus transfer between cash and** 163 **fingertips**

164 Experiments performed under controlled laboratory conditions demonstrated the  
165 persistence of SARS-CoV-2 on inanimate surfaces for days and consequently implied  
166 the risk of viral transmission via contaminated objects (Chin et al. 2020; van Doremalen  
167 et al. 2020). However, to develop more refined models to assess the risk of fomites-based  
168 transmission of SARS-CoV-2, quantitative measurements of the transfer efficiency of  
169 infectious virus between skin and surfaces are required. To address these limitations, we  
170 developed a touch transfer assay to study the transfer of infectious BCoV and SARS-  
171 CoV-2 between fingertips and different fomites (Figure 3). Briefly, virus suspensions  
172 were placed on different surfaces (pieces of 10 € banknotes, 10 cent coins, pieces of PVC  
173 to mimic the surface of credit cards and stainless-steel discs as reference material).  
174 Afterwards, the wet inoculum or the dried suspension was touched by “printing” or  
175 “rubbing” using fingertips (BCoV) or an artificial skin fabric (SARS-CoV-2) (Figure 3).  
176 Subsequently, infectious viruses were recovered by dipping and rubbing each fingertip  
177 in turn for one minute on the base of a Petri dish containing 2 mL of EMEM cell culture

178 medium (BCoV) or, in case of the artificial skin, by directly placing it into a container  
179 with cold DMEM (SARS-CoV-2). The resulting suspension was serially diluted to  
180 determine TCID<sub>50</sub>/mL values of the remaining infectious virus.

181

### 182 **Transferability of BCoV from banknotes, coins and PVC to fingertips**

183 Using this newly developed touch transfer assay, we examined the transmission of BCoV  
184 from different surfaces, i.e. pieces of 10 € banknotes, 10 cent coins, pieces of PVC and  
185 stainless-steel discs as reference material, to fingertips. Surfaces were inoculated with  
186 either a high ( $\sim 1 \times 10^6$  TCID<sub>50</sub>/mL) or low ( $\sim 1 \times 10^4$  TCID<sub>50</sub>/mL) viral titer to represent  
187 different degrees of surface contamination. Virus transfer was assessed directly  
188 following application to fomites (wet) or after  $\sim 1$  h until completely dried (dry) by either  
189 pressing (print) or rubbing (rub) the fingertip onto the surface. Initial virus (input) was  
190 determined by applying the fomites directly to the medium container. For a high viral  
191 load and direct surface contact, we observed a maximum of a 0.6 log<sub>10</sub> reduction for the  
192 10-cent coin and 10 € banknote, while lower reduction factors were observed for the  
193 other surfaces (Figure 4A). In case of drying the initial inoculum followed by a  
194 fingerprint, we observed a 2.1 log<sub>10</sub> reduction on a 10 € banknote, while lower reduction  
195 factors were observed for the other surfaces. For a low initial titer and direct surface  
196 contact, we observed the highest reduction on the stainless-steel carrier (1.2 log<sub>10</sub>  
197 reduction). In case of drying the initial inoculum followed by a fingerprint, we observed  
198 a 0.8 log<sub>10</sub> reduction on a 10-cent coin. Importantly, no infectious virus could be  
199 recovered from the 10 € banknote under these conditions.

200

### 201 **Transferability of SARS-CoV-2 from banknotes, coins and PVC to fingertips**

202 Next, we examined the transmission of infectious SARS-CoV-2 from surfaces to  
203 fingertips. Surfaces were inoculated with either a high ( $\sim 1 \times 10^6$  TCID<sub>50</sub>/mL) or low ( $\sim$   
204  $1 \times 10^4$  TCID<sub>50</sub>/mL) titer to represent different degrees of surface contamination. As  
205 described before, virus transfer was assessed directly following inoculation (wet) or after  
206 drying either by printing (print) or rubbing (rub) the fingertip onto the surface. For a high  
207 initial titer and direct surface contact, we observed a maximum of a 1 log<sub>10</sub> reduction for  
208 the 10-cent coin, while lower reduction factors were observed for the other surfaces  
209 (Figure 5A). Drying of the initial inoculum led to  $\sim 1$  log loss in virus titer. In the dried  
210 state, less virus was transferred and could be recovered, e.g. by printing the fingertip we  
211 observed a 3.0 log<sub>10</sub> reduction on the 10-cent coin, while lower reduction factors were  
212 observed for the other surfaces. For a low initial titer and direct surface contact, we  
213 observed the highest reduction on the 10 € banknote (0.7 log<sub>10</sub> reduction). In case of  
214 drying the initial inoculum followed by a fingerprint we observed a reduction of the  
215 initial inoculum after 1 h desiccation to close/under the limit of quantification and only  
216 from the PVC very low ( $2.19 \times 10^1$  TCID<sub>50</sub>/mL) amounts of infectious virus could be  
217 recovered (Figure 5B).

218

**219 Discussion**

220

221 Human-to-human transmission of SARS-CoV-2 occurs primarily by respiratory aerosols  
222 or droplets and subsequent contact to nasal, oral, or ocular mucosal membranes. Based  
223 upon virus stability on surfaces, fomite transmission of SARS-CoV-2 has been  
224 considered possible (Chin et al. 2020; van Doremalen et al. 2020; Biryukov et al. 2020;  
225 Bueckert et al. 2020; Kwon et al. 2021; Riddell et al. 2020), however, the importance of  
226 this route in healthcare and public settings remains controversial (Goldman 2020;  
227 Mondelli et al. 2020; Pitol and Julian 2021). Fomite-based transmission has been  
228 proposed to contribute to the spread of other common respiratory pathogens (Kraay et  
229 al. 2018; Boone and Gerba 2007). Consequently, paper currency and coins have been  
230 suspected as a potential transmission vehicle for various pathogens, including SARS-  
231 CoV-2 (Pal and Bhadada 2020; Angelakis et al. 2014; Xiao et al. 2017). Although  
232 infectious viruses have not been directly detected on banknotes or coins, the potential  
233 for their transmission has been proposed because of the observation that human influenza  
234 viruses were able to persist and remain infectious for several days when they were  
235 deposited on banknotes (Thomas et al. 2008). Furthermore, many other viruses, (i.e.  
236 Adenoviruses, Rotaviruses) are stable in the environment and exhibit high infectivity  
237 and, thus, could possibly be transferred by banknotes and coins (Wißmann et al. 2021).

238 In agreement with previous reports we found that high titers of SARS-CoV-2 and its  
239 surrogate BCoV, after an initial loss of infectivity, remained infectious for days under  
240 laboratory conditions on banknotes and coins (Table 1, Figure 1 and 2) (Harbourt et al.  
241 2020; Chin et al. 2020). The initial loss of infectivity was higher on coins and banknotes,  
242 irrespective of protective varnish, when compared to stainless steel, indicating faster

243 desiccation due to liquid absorption (banknotes) or antiviral surface properties (e.g.  
244 copper in coins). Both BCoV and SARS-CoV-2 displayed highly comparable levels of  
245 virus transfer and stability among the different conditions (Figure 6), implying that  
246 BCoV is also a suitable surrogate virus to model surface transmission of SARS-CoV-2.

247 Decay of SARS-CoV2 is likely determined by a combination of the initial amount of  
248 infectious virus deposited on a given surface and other environmental parameters  
249 (temperature, humidity, media components, light and UV conditions). For example, the  
250 reduction of viral titers after drying was lower for the high viral load compared to the  
251 low viral load samples, indicating a dose-dependent effect of the viral decay after drying.

252 Interestingly, this dose-dependency is in line with findings for survival of SARS-CoV-1  
253 on paper and cotton (Lai et al. 2005). Furthermore, persistence of pathogens in the  
254 environment represents only the first requirement for self-inoculation via contaminated  
255 fingers. However, the possibility of fingerprint transmission has quantitatively been  
256 examined only in the context of bacteria (Knobloch et al. 2017; Chen et al. 2001). Using  
257 a newly developed virus touch transfer assay, we observed that the transfer of BCoV and  
258 SARS-CoV-2 between fomites and fingertips is context-dependent: For a high initial  
259 virus titer ( $\sim 10^6$  TCID<sub>50</sub>/mL), the transfer was more efficient for the wet inoculum, while  
260 visual desiccation on the one hand resulted in reduction of the titer as outlined above, as  
261 well as less efficient mobilization of the viral particles, reflected by higher reduction  
262 factors. Consequently, lower viral burdens ( $\sim 10^4$  TCID<sub>50</sub>/mL) mimicking more realistic  
263 real life contamination events, as observed for influenza viruses in aerosol particles from  
264 human coughs (Lindsley et al. 2010; Goldman 2020), were not effectively transferred  
265 (Figure 4 and 5). Recent studies estimated a minimal infectious dose of SARS-CoV-2 in  
266 the range of  $3 \times 10^2$  to  $2 \times 10^3$  viral particles (Popa et al. 2020; Basu 2021), or as low as

267 100 particles (Karimzadeh et al. 2021). Overall, our results point to a low risk of SARS-  
268 CoV-2 transmission by coins and banknotes and the rush to abandon cash during the  
269 pandemic seems unnecessary.

270 Given that cash is typically stored securely in wallets and purses, the risk of direct  
271 contamination through exhaled droplets and aerosols seems much lower than constantly  
272 exposed surfaces (e.g. doorbell, shopping carts). The role of a contagious person  
273 contaminating banknotes and coins afresh when handing over, needs to be addressed in  
274 future studies. Current government regulations to wear masks minimize the spread of  
275 exhaled droplets and aerosols, and in combination with good hand hygiene also mitigate  
276 the risk of transmission via contaminated surfaces. Still, contamination of cash is most  
277 likely to occur indirectly by transfer from the hands of an infected person or finger  
278 contact with a contaminated surface. However, any contamination by these routes would  
279 likely result in a much lower degree of surface contamination than by direct  
280 contamination as investigated in this study. Current literature suggests that inanimate  
281 surfaces are neglectable as sources for SARS-CoV-2 transmission (Goldman 2020;  
282 Harvey et al. 2021; Kampf et al. 2021). Consequently, the overall chance of transmission  
283 of SARS-CoV-2 through banknotes, coins and credit/debit cards seems low since a  
284 timely order of specific events is required – sufficient viable virus deposited on a surface,  
285 survival of the virus until the surface is touched, transfer of an infectious dose of virus  
286 to fingers, and transfer from fingers without washing hands to mouth, nose or eyes.

287

288

289

**290 Limitations:**

291 The following limitations of this study have to be considered. *In vitro* studies can provide  
292 a first indication to assess the risk of transmission of a particular pathogen. However, the  
293 conditions in a controlled laboratory environment, as herein presented, frequently not do  
294 resemble real-life scenarios (i.g., large inoculums, small surface area UV exposure etc.),  
295 necessitating careful interpretation. For example, a study by Harbourt et al. reported a  
296 temperature-dependent stability of SARS-CoV-2 infectivity on banknotes and detected  
297 for at least 8 h at 22°C and longer at 4°C (Harbourt et al. 2020). In addition, our  
298 experiments were performed with lab-grown viruses in permissive eukaryotic cells and  
299 might therefore not recapitulate the specific infectivity of patient-derived SARS-CoV-2  
300 particles. In particular additional patient-specific factors (i.e. mucus and/or saliva) and  
301 cell culture-specific factors (i.e. FBS/BSA) of the prepared inocula and their respective  
302 impacts on the viral stability can influence experimental outcomes. Likewise, the  
303 artificial skin employed in this study might not completely resemble the composition of  
304 real human skin.

305 In a worst-case scenario including high virus loads (directly coughing or sneezing on the  
306 coin or banknote) and high transfer efficiencies (wet body liquid) with neglectable  
307 inactivation by desiccation (immediate money transfer), SARS-CoV-2 transmission  
308 might be possible. While our results clearly show that SARS-CoV-2 can be transferred  
309 from banknotes/coins to finger tips under certain conditions (large inoculum and wet  
310 transfer), an additional step is required to transfer the virus to the respiratory system:  
311 transfer from fingertips to the nose or mouth and respective mucosal surfaces. A  
312 quantitative microbiological risk assessment (QMRA) of SARS-CoV-2 transmission in

313 real life via banknotes/coins should consider these steps and relate the amount of  
314 remaining infectious virus to the human infectious dose.

315

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324

325 **Author contributions**

326 Conceptualization: DT, BT, JH, FHB, JST, SP, ES. Data curation: DT, TLM, DP, BB,  
327 MW, JS. Formal Analysis: DT, JS. Funding acquisition: BT, JH, ES. Investigation:  
328 TLM, DP, BB, NH, VK, BM, Methodology: DT, FHB, SP, ES. Project administration:  
329 BT, JH, FHB, ES. Resources: FHB, BM, CG, AK, JST, SP, ES. Validation: DT, TLM,  
330 DP, BB, FHB, MW, JS, ES. Visualization: DT, YB. Writing – original draft: YB, SP,  
331 ES. Writing – review & editing: all authors.

332

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335

336 **Figure legends**

337 **Figure 1: Stability of BCoV on banknotes and steel discs.** BCoV stock solution was  
338 applied on 2 cm × 2 cm pieces of 10 € or 50 € banknotes and recovered after the indicated  
339 times. Residual titer was assessed via limiting dilution assay. Temperature during  
340 experiments was logged ( $18\text{ °C} \pm 1\text{ °C} - 25\text{ °C} \pm 1\text{ °C}$ ) **A)** Infectious BCoV recovered,  
341 displayed as raw TCID<sub>50</sub>/mL (y-axes) over time (categorical x-axes). Dots indicate mean  
342 values of three independent experiments with standard deviation, lower limit of  
343 quantification (LLOQ) is shown as dashed line. **B)** Recovered BCoV displayed as  
344 TCID<sub>50</sub>/mL (y-axes) over time (continuous x-axes). Dots represent individual biological  
345 experiments, purple lines and areas display the course of the Weibull distribution fitted  
346 data and 95% confidence interval, LLOQ is shown as dashed line. Virus particles created  
347 with BioRender.com.

348

349 **Figure 2: Stability of SARS-CoV-2 on banknotes, coins and steel discs.** SARS-CoV-  
350 2 stock solution was applied on 2 cm × 2 cm pieces of 10 € banknotes, 1 €, 10 cent and  
351 1 cent coins and recovered after the indicated times. Residual titer was assessed via  
352 limiting dilution assay. Humidity and temperature during experiments was logged  
353 (32% - 43% RH,  $22.4\text{ °C} - 23.2\text{ °C}$ ) **A)** Infectious SARS-CoV-2 recovered, displayed as  
354 raw TCID<sub>50</sub>/mL (y-axes) over time (categorical x-axes). Dots indicate mean values of  
355 three independent experiments with standard deviation, lower limit of quantification  
356 (LLOQ) is shown as dashed line. **B)** Recovered SARS-CoV-2 displayed as TCID<sub>50</sub>/mL  
357 (y-axes) over time (continuous x-axes). Dots represent individual biological  
358 experiments, green lines and areas display the course of the Weibull distribution fitted

359 data and 95% confidence interval, LLOQ is shown as dashed line. Virus particles created  
360 with BioRender.com.

361

362 **Figure 3: Touch transfer assay setup.** To study the transfer of infectious BCoV and  
363 SARS-CoV2 between fingertips and different fomites, 50  $\mu$ L virus suspensions are  
364 placed on different surfaces (pieces of 10 € banknotes, 10 cent coins, pieces of PVC to  
365 mimic the surface of credit cards and stainless-steel discs as reference) in 10  $\mu$ L spots.  
366 Afterwards, the wet inoculum or the dried suspension is touched by “printing” or  
367 “rubbing” using fingertips (BCoV) or an artificial skin fabric (SARS-CoV-2).  
368 Subsequently, infectious virus was recovered by rubbing the fingertip on the bottom of  
369 a petri dish filled with respective culture media or in case of the artificial skin directly  
370 transferred into a container. The resulting suspension is serially diluted to determine  
371 TCID<sub>50</sub>/mL values of the remaining infectious virus. Virus particles created with  
372 BioRender.com.

373

374 **Figure 4: Transferability of BCoV from cash fomites to fingertips.** Bars depict titer  
375 of input virus suspension and recovered infectious virus from different cash fomites, i.e.  
376 10 cent coin, 10 € banknote, PVC and steel disc carrier in four different scenarios; mean  
377  $\pm$  SD. Temperature during experiments was logged (18 °C  $\pm$  1 °C – 25 °C  $\pm$  1 °C) **A)**  
378 High initial input titer ( $\sim 10^6$  TCID<sub>50</sub>/mL) wet, when directly touch after application and  
379 dry, when transferred after visual desiccation and **B)** low initial input titer ( $\sim 10^4$   
380 TCID<sub>50</sub>/mL), wet and dry. Each scenario was performed by three test persons using eight  
381 fingers each. Numbers above bars indicate reduction factor, lower limit of quantification  
382 (LLOQ) is shown as dashed line.

383 **Figure 5: Transferability of SARS-CoV-2 from cash fomites to fingertips.** Bars  
384 depict titer of input virus suspension and recovered infectious virus from different cash  
385 fomites, i.e. 10 cent coin, 10 € banknote, PVC and steel disc carrier in four different  
386 scenarios; mean  $\pm$  SD. Humidity and temperature during experiments was logged  
387 (32% - 43% RH, 22.4 °C – 23.2 °C) **A)** High initial input titer ( $\sim 10^6$  TCID<sub>50</sub>/mL) wet,  
388 when directly touch after application and dry, when transferred after visual desiccation  
389 and **B)** low initial input titer ( $\sim 10^4$  TCID<sub>50</sub>/mL), wet and dry. Numbers above bars  
390 indicate reduction factor, lower limit of quantification (LLOQ) is shown as dashed line.  
391 Virus particles created with BioRender.com.

392  
393 **Figure 6: Suitability of BCoV as surrogate for SARS-CoV-2 in touch transfer**  
394 **studies.** Titers of recovered infectious virus were matched between BCoV and SARS-  
395 CoV-2 for each scenario and linear regression curves calculated for input, rub and print.  
396 Gray line and area represent the overall linear regression and 95% confidence interval  
397 of all matched data points, dashed line depicts perfect correlation.

398

399 **STAR METHODS**

400

401 **RESOURCE AVAILABILITY**

402

403 **Lead contact**

404 Further information and requests for resources should be directed to and will be fulfilled  
405 by the lead contact, Eike Steinmann, Ruhr University Bochum, Germany  
406 (eike.steinmann@rub.de).

407

408 **Materials availability**

409 This study did not generate new unique materials.

410

411 **Data and code availability**

412 All data produced or analyzed for this study are included in the published article and its  
413 supplementary information files. This paper does not report original code. Any  
414 additional information required to reanalyze the data reported in this paper is available  
415 from the lead contact upon request.

416

417

**418 METHOD DETAILS**

419

**420 Preparation of test virus suspension**

421 For preparation of SARS-CoV-2 test virus suspension, Vero E6 cells (kindly provided  
422 by C. Drosten and M. Müller – Charité, Germany) were seeded in 75 cm<sup>2</sup> flasks at 2×10<sup>6</sup>  
423 cells in Dulbecco's Modified Eagle's Medium (DMEM, supplemented with 10 % (v/v)  
424 fetal calf serum (FCS), 1 % non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL  
425 streptomycin and 2 mM L-Glutamine). The monolayer was either inoculated with hCoV-  
426 19/Germany/BY-Bochum-1/2020 (B.1.1.70) (GISAID accession ID:  
427 EPI\_ISL\_1118929), which was isolated during the first wave in Northern Europe and  
428 closely resembles the original Wuhan outbreak strain harboring a G614D mutation in  
429 the spike protein, or alpha variant (RKI-0026\_B.1.1.7) (GISAID accession ID:  
430 EPI\_ISL\_751799). Strains were checked for lineage specific features as described in the  
431 supplement to Meister et al. 2021 (Meister et al. 2021). After 3 days and upon visible  
432 cytopathic effect the supernatant was harvested by centrifugation at 1,500 rpm for 5 min  
433 at room temperature, aliquoted and stored at -80 °C until further usage.

434 For preparation of BCoV virus suspension, U373 cells were cultivated in a 75 cm<sup>2</sup> flask  
435 in Minimum Essential Medium Eagle (EMEM) supplemented with L-glutamine, non-  
436 essential amino acids, sodium pyruvate and 10 % FCS. Before virus infection, cells were  
437 washed two times with phosphate buffered saline (PBS), incubated for 3 h with serum-  
438 free EMEM and were washed once with EMEM supplemented with trypsin. For virus  
439 production, BCoV strain L9 (NCBI: txid11130) was added to the prepared monolayer.  
440 After an incubation period of 24 to 48 h cells were lysed by a rapid freeze/thaw cycle  
441 followed by a low speed centrifugation in order to sediment cell debris. After aliquoting

442 of supernatant, test virus suspension was stored at  $-80\text{ }^{\circ}\text{C}$ . For assays, nine volumes of  
443 test virus suspension were mixed with one volume of interfering substance solution (final  
444 concentration of  $0.3\text{ g/L}$  bovine serum albumin (BSA) in PBS) according to European  
445 Testing guideline (EN 16777, section 5.2.2.8). The tests were performed with two  
446 different virus concentrations, i.e. a titer of approximately  $10^4$  50% tissue culture  
447 infectious dose per milliliter ( $\text{TCID}_{50}/\text{mL}$ ) and a titer of  $10^6$   $\text{TCID}_{50}/\text{mL}$  corresponding  
448 to an absolute viral load of approximately  $5 \times 10^2$  and  $5 \times 10^4$   $\text{TCID}_{50}$ , respectively.

449

#### 450 **Preparation of specimens**

451 Prior to use regular, 5-, 10-cent and 1-euro coins were dipped in a bath containing 70 %  
452 (v/v) ethanol for 5 min. The 10- and 50-euro banknotes (provided by the European  
453 Central Bank) and PVC plates [with PUR (polyurethane) surface coating 20 x 50 cm  
454 (VAH e.V.), precleaned with 70.0 % propan-1-ol or ethanol] were cut into pieces of 2 x  
455 2 cm. Banknotes were UV irradiated before the tests. Stainless steel discs (2 cm diameter  
456 discs) with Grade 2 B finish on both sides (article no. 4174-3000, GK Formblech GmbH,  
457 Berlin, Germany) served as reference control. Prior to use, discs were decontaminated  
458 with 5 % (v/v) Decon 90 for 60 minutes and 70 % (v/v) propan-2-ol for 15 min.  
459 Subsequently, the discs were rinsed with distilled water sterilized by autoclaving (steam  
460 sterilization).

461

#### 462 **Inactivation assays and controls**

463 For stability testing, specimens were placed aseptically in a Petri dish and inoculated  
464 with  $50\text{ }\mu\text{L}$  of the virus inoculum [ $5 \times 10\text{ }\mu\text{L}$  drops, i.e. four in every corner and one in  
465 the middle of the square]. After visible drying of the inoculum, the petri dishes were

466 closed and the specimens were incubated until the end of the appropriate exposure time  
467 (up to 7 days). All experiments were performed at room temperature ( $18\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  to  $25$   
468  $^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ ) and a relative humidity in the range of 30-45%. After the respective time, the  
469 specimens were transferred to 2 mL cell culture medium (without FCS) in a 25 mL  
470 container and vortexed for 60 seconds to resuspend the virus. Directly after elution,  
471 series of ten-fold dilutions of the eluate in ice-cold maintenance medium were prepared  
472 and inoculated on cell culture. Final concentrations of interfering substances when  
473 applied to cells in first wells of TCID<sub>50</sub> assay was 7.5 mg/L BSA and 0.225% FCS.  
474 Fifteen and 30 minutes, 1, 2, 7, and 24 hours and 2, 3, 5 and 7 days were chosen as  
475 application times. Eluates were retained after appropriate drying times and residual  
476 infectivity was determined.

477 The initial virus titer was determined by addition of 50  $\mu\text{L}$  of the virus inoculum directly  
478 to 2 mL cell culture medium without any desiccation.

479

#### 480 **Touch transfer test**

481 For the touch transfer test with BCoV, three test persons simulated the transfer by  
482 pressing a finger shortly on the dried inoculum on the respective carriers followed by  
483 rubbing once with pressure over the carrier. Virus transfer was either assessed directly  
484 following application to fomites (wet) or after  $\sim 1$  h until desiccation time (dry). Three  
485 other test persons simulated the transfer by a fingerprint of 5 seconds on the dried  
486 inoculum on the different carriers. Each test person performed the transfer test separately  
487 with the two different virus concentrations ( $10^4$  TCID<sub>50</sub>/mL and  $10^6$  TCID<sub>50</sub>/mL) with 8  
488 fingers each. For each test person and virus concentration, two fingers were used for

489 virus transfer without drying of the inoculum. The transfer procedure was the same as  
490 with the dried inoculum, i.e. after visual desiccation.

491 The amount of transferred virus to the fingers was obtained by dipping and rubbing each  
492 finger in turn for one minute on the base of a Petri dish containing 2 mL cell culture  
493 medium without FCS as sample fluid. For each finger a separate dish was used. The  
494 eluates were transferred in a 25 mL container. Directly after elution, series of ten-fold  
495 dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on  
496 cell culture. The initial virus titer was determined by addition of 50  $\mu$ L of the virus  
497 inoculum directly to 2 mL cell culture medium without any drying. Furthermore, a cell  
498 control (only addition of medium) was incorporated.

499 For the touch transfer test of SARS-CoV-2, one person performed all assays due to BSL3  
500 restrictions. To mimic the texture and nature of human fingertips, we used VITRO-SKIN  
501 (IMS Florida Skincare Testing, FL, USA), an artificial skin substitute, placed in a plastic  
502 frame. Virus transfer was either assessed directly following application to fomites (wet)  
503 or after  $\sim$  1 h until desiccation time (dry). After printing or rubbing as described above  
504 (here three replicates), the complete artificial skin was released from the frame and  
505 transferred into a 25 mL container with serum-free cell culture medium and vortexed for  
506 60 s. All experiments were performed at room temperature ( $18\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  to  $25\text{ }^{\circ}\text{C} \pm 1$   
507  $^{\circ}\text{C}$ ) and a relative humidity in the range of 30-45%.

508 Respective input virus titers were determined on separate specimens directly before  
509 transfer.

510

511

512

## 513 **QUANTIFICATION AND STATISTICAL ANALYSIS**

514

### 515 **Determination of infectivity**

516 Infectivity was determined by means of end point dilution titration using the microtiter  
517 process. For this, samples were immediately diluted at the end of the exposure time with  
518 ice-cold EMEM containing trypsin and 100  $\mu$ L of each dilution were placed in 6 or 8  
519 wells of a sterile polystyrene flat-bottomed plate with a preformed U373 (BCoV) or Vero  
520 E6 (SARS-CoV-2) monolayer. Before addition of virus, cells were washed twice with  
521 EMEM (U373) or DMEM (Vero E6) and incubated for 3 h with 100  $\mu$ L EMEM (U373)  
522 or DMEM (Vero E6) with trypsin. After 3 d or 6 d incubation at 37 °C in a CO<sub>2</sub>-  
523 atmosphere (5.0 % CO<sub>2</sub>-content), cultures were observed for cytopathic effects.  
524 TCID<sub>50</sub>/mL was calculated according to the method of Spearman and Kärber (Wulff et  
525 al. 2012). Lower limit of quantification (LLOQ) was defined as theoretical titer which  
526 yields in all wells for the lowest virus dilution being positive, while all others are  
527 negative (prerequisite for reliable application of method of Spearman is all wells should  
528 be positive at least for the lowest virus dilution) (Vieyres and Pietschmann 2013).

529

### 530 **Fitting of virus titer decay**

531 To account for different virus decay during desiccation and under wet incubation  
532 conditions, we implemented a Weibull distribution fit in GraphPad Prism version 9.0.2  
533 for Windows (GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com)).  
534 Only time points with residual viral titers of at least one replicate above the LLOQ were  
535 used for modelling. We used stock virus titers as initial titers for modelling to account  
536 for rapid loss due to inactivation/desiccation.

537 **Calculation of the reduction factor**

538 The loss in virus titer by desiccation was calculated by subtracting the  $\log_{10}$  titer on the  
539 different carriers after desiccation from the  $\log_{10}$  titer of the initial virus control. The  
540 amount of transferred virus ( $\text{TCID}_{50}/\text{mL}$ ) from different carriers to fingers was also  
541 calculated with the method of Spearman and Kärber (Wulff et al. 2012).

542

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544 **Declaration of Interests**

545 Daniel Todt receive consulting fees from the European Central Bank. Eike Steinmann

546 receive consulting fees from the European Central Bank and is a member of its

547 scientific advisory board of Dr. Brill + Partner GmbH. Florian H. Brill is executive

548 partner of Dr. Brill + Partner GmbH.

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677 Tully/Alex D. Washburne/Tom Wenseleers/Amy Gimma/William Waites/Kerry  
678 L. M. Wong/Kevin van Zandvoort/Justin D. Silverman/CMMID COVID-19  
679 Working Group<sup>1</sup>‡/COVID-19 Genomics UK (COG-UK) Consortium<sup>‡</sup>/Karla  
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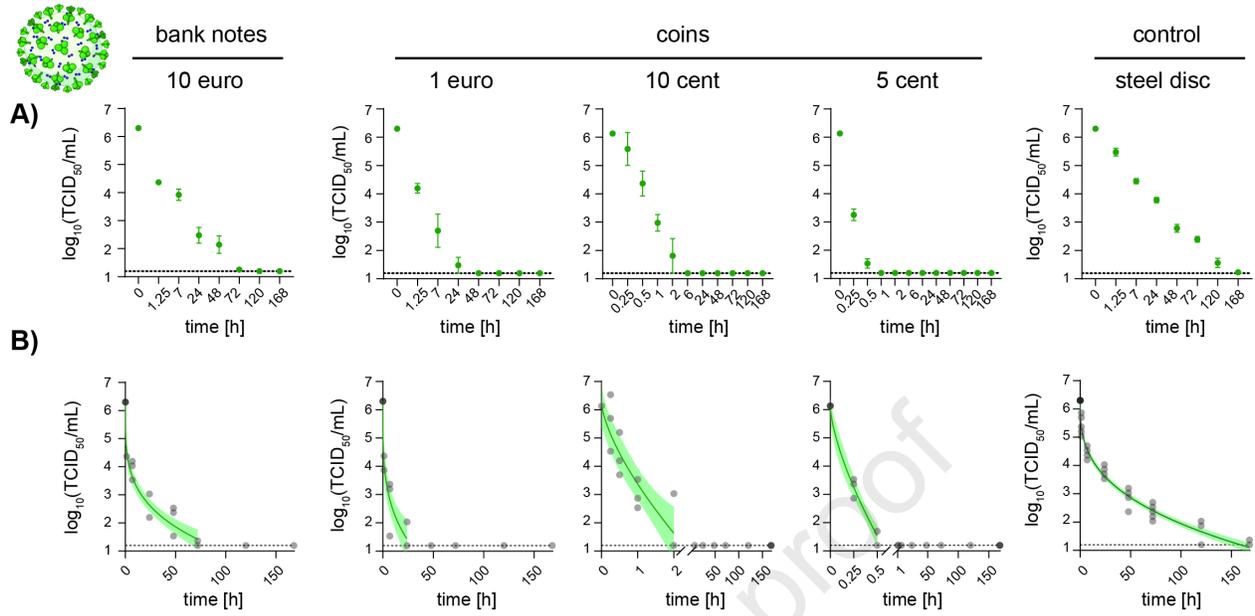
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699 Michael/Grau, Miguel L./Martínez-Jiménez, Francisco/Pich, Oriol/Borena,  
700 Wegene/Pawelka, Erich/Keszei, Zsofia/Senekowitsch, Martin/Laine, Jan/Aberle,  
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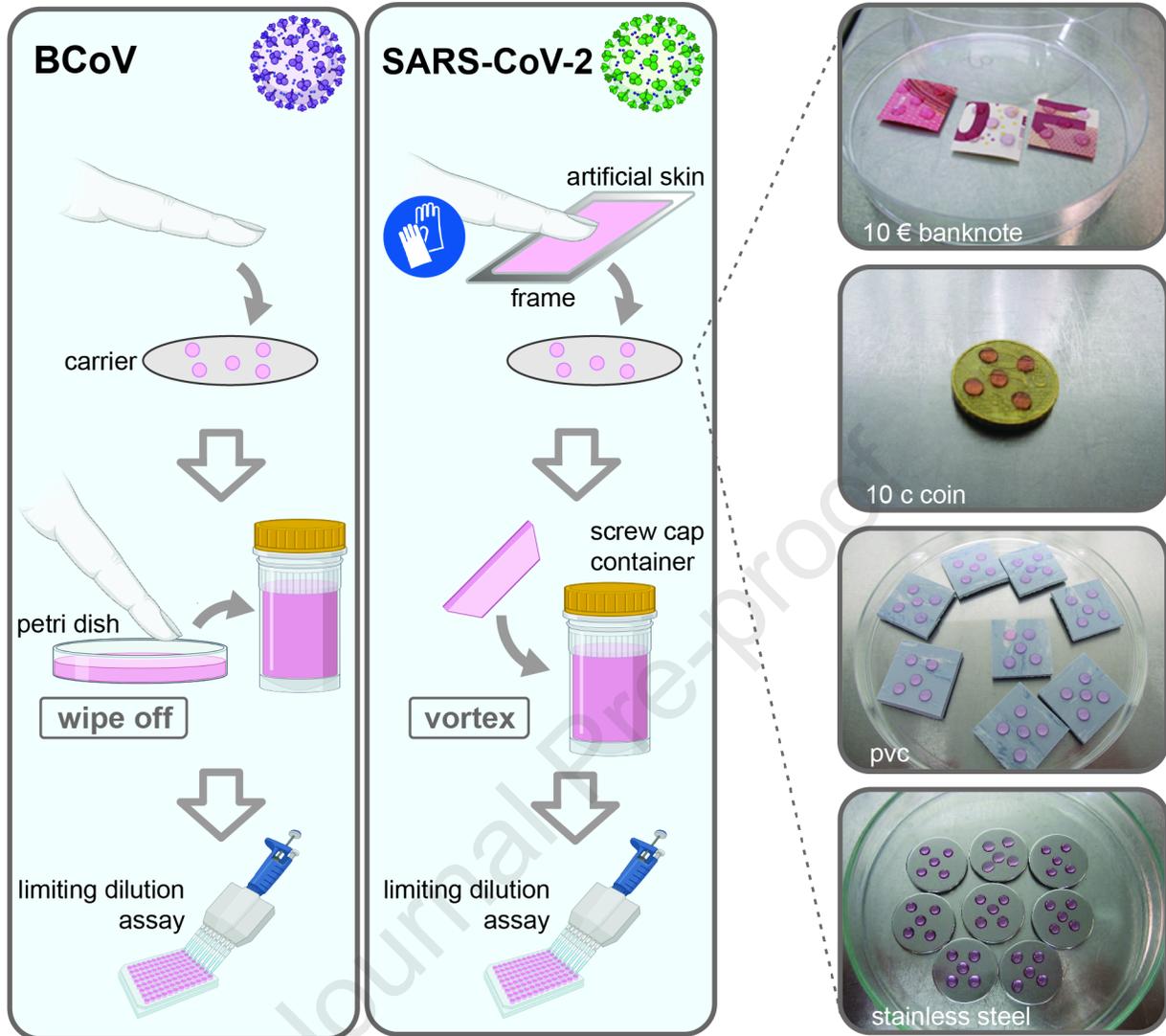
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Table 1: Initial decay time and time to reach lower limit of quantification (LLOQ) calculated from modelled curves.

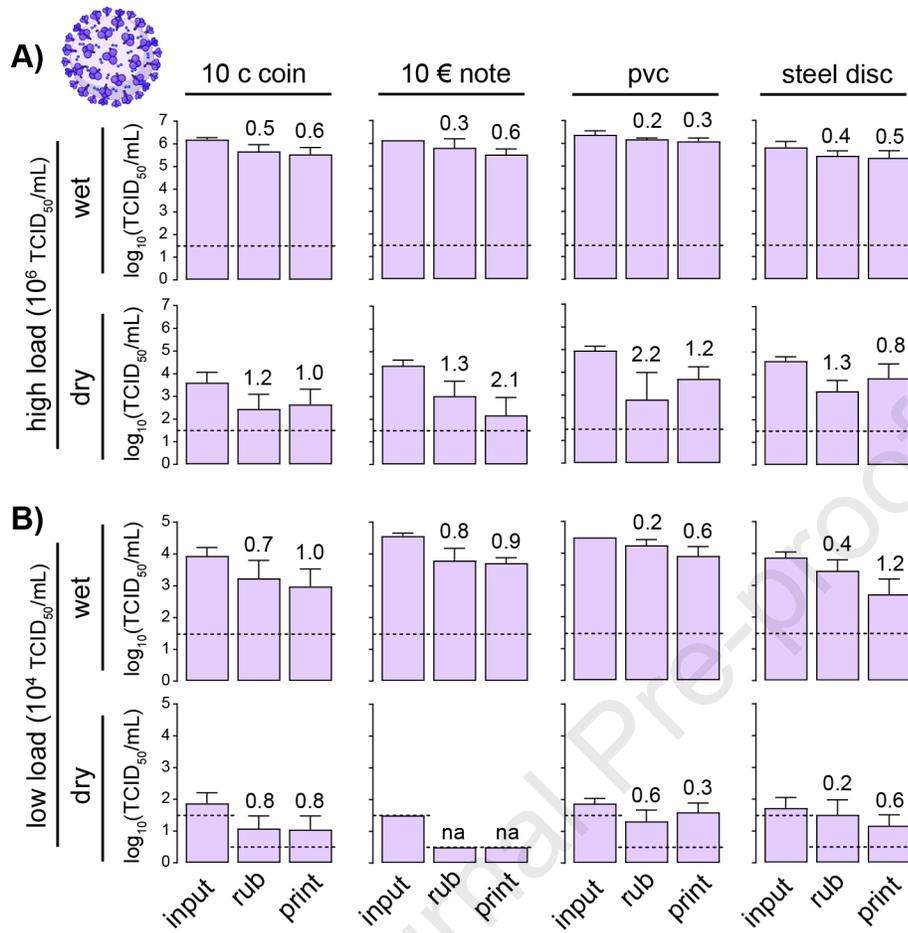
		SARS-CoV-2				BCoV	
		B.1.1.70 (wild type)		B.1.1.7 (alpha)			
	material	Initial decay [h]	time to LLOQ [h]	Initial decay [h]	time to LLOQ [h]	initial decay [h]	time to LLOQ [h]
notes	50 euro					2.8	175.6
	10 euro	6.1	85.7	0.22	59.23	6.5	216.3
coins	1 euro	2.2	28.4	0.74	70.72		
	10 cent	0.8	2.3	0.49	37.07		
	5 cent	0.2	0.6	0.12	2.25		
control	steel disc	20.6	158.8	1.21	882.92	53.5	240.2

## SARS-CoV-2

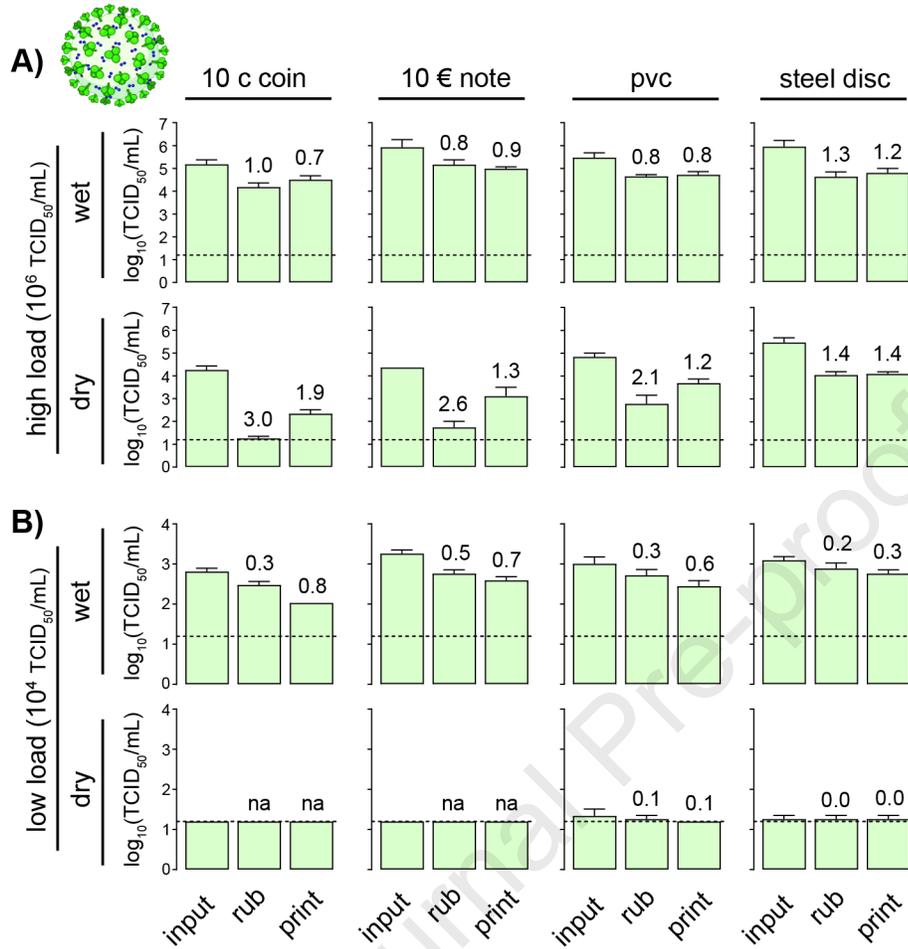


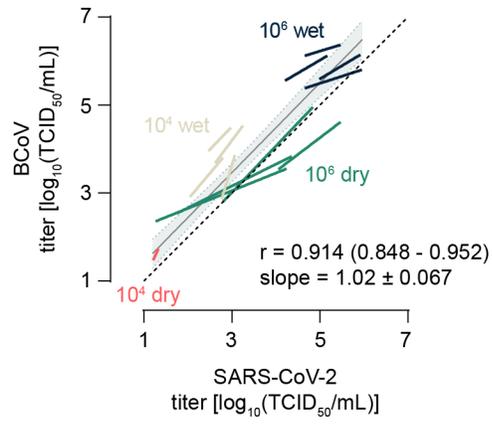


## BCoV

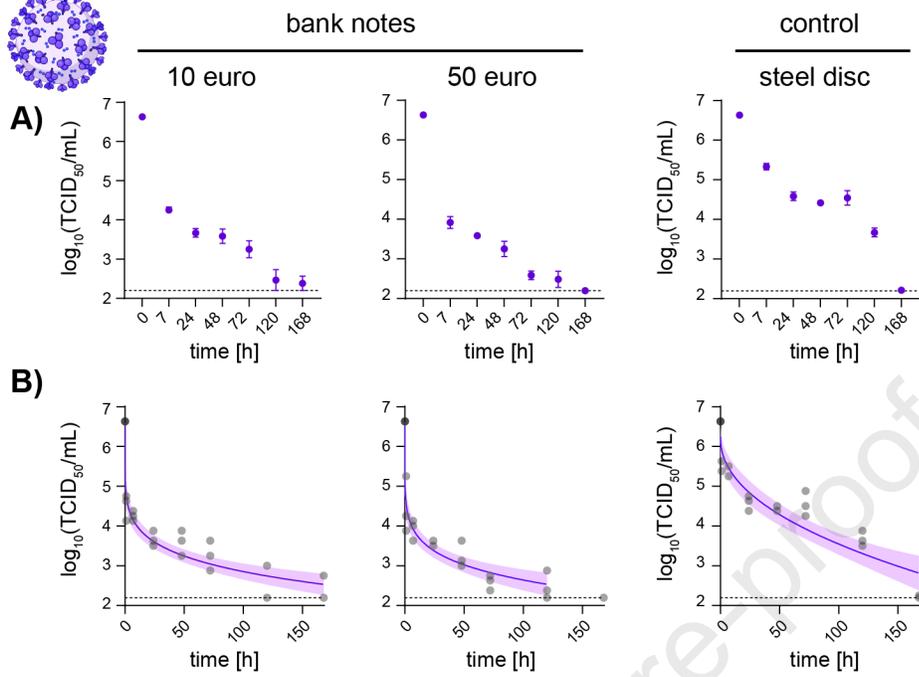
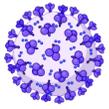


## SARS-CoV-2





## BCoV



### **Highlights**

- Paper currency and coins could be potential transmission vehicles for SARS-CoV-2.
- High titers of SARS-CoV-2 remained infectious for days on banknotes and coins.
- Transmission to fingers is context dependent in a novel virus touch-transfer model.
- Chance of transmission through banknotes, coins and credit/debit cards is unlikely.

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