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

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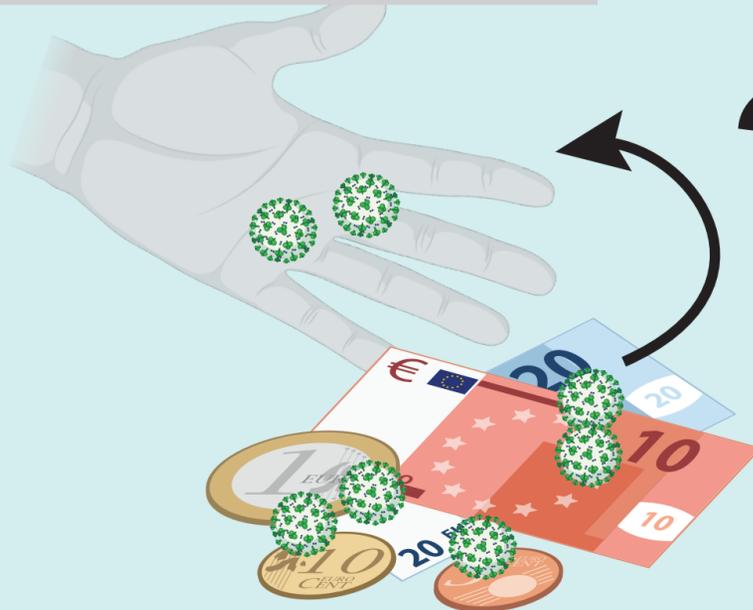
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stability

Journal Pre-proof

touch-transfer



Stability and touch-transfer of SARS-CoV-2



desiccation



viral titer



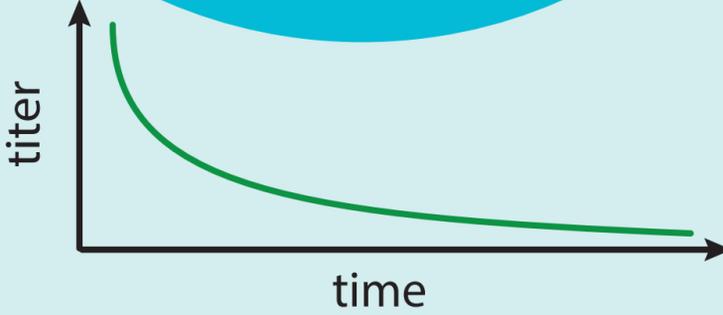
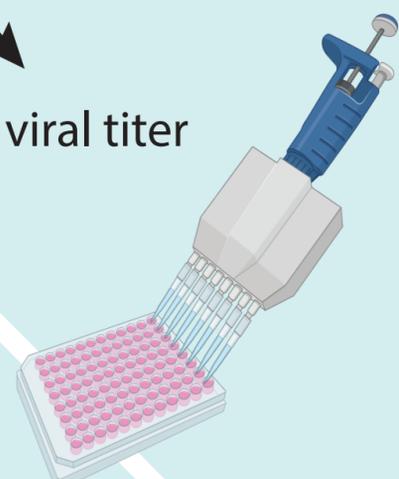
wet transfer



transfer after desiccation



viral titer



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 ($\text{TCID}_{50}/\text{mL}$)) on the tested surfaces, while lower initial virus titer stocks (10^4
101 $\text{TCID}_{50}/\text{mL}$) 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 4.3×10^6 TCID₅₀/mL declined to 1.84×10^4 TCID₅₀/mL on 10 €
119 banknotes and 9.25×10^4 TCID₅₀/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 $1.36 - 2.0 \times 10^6$ TCID₅₀/mL (Figure 2). On 10 € banknotes
135 and 1 € coins, the initial virus concentration declined to 2.32×10^4 TCID₅₀/mL and 1.79
136 $\times 10^4$ TCID₅₀/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 5.96×10^4 TCID₅₀/mL and 3.86×10^1 TCID₅₀/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 6.87×10^6 TCID₅₀/mL (Figure S1). On all surfaces, the initial virus
152 concentration declined to $\sim 5 - 1 \times 10^4$ TCID₅₀/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 TCID₅₀/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 ($\sim 1 \times 10^6$ TCID₅₀/mL) or low ($\sim 1 \times 10^4$ TCID₅₀/mL) viral titer to represent
187 different degrees of surface contamination. Virus transfer was assessed directly
188 following application to fomites (wet) or after ~ 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 log₁₀ 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 log₁₀ 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 log₁₀
197 reduction). In case of drying the initial inoculum followed by a fingerprint, we observed
198 a 0.8 log₁₀ 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 ($\sim 1 \times 10^6$ TCID₅₀/mL) or low (\sim
204 1×10^4 TCID₅₀/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 log₁₀ 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 ~ 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 log₁₀ 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 log₁₀ 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 (2.19×10^1 TCID₅₀/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 ($\sim 10^6$ TCID₅₀/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 ($\sim 10^4$ TCID₅₀/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 3×10^2 to 2×10^3 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:
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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 TCID₅₀/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 TCID₅₀/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 TCID₅₀/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 TCID₅₀/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 μ 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 μ 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 TCID₅₀/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 ($\sim 10^6$ TCID₅₀/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 TCID₅₀/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 ($\sim 10^6$ TCID₅₀/mL) wet,
388 when directly touch after application and dry, when transferred after visual desiccation
389 and **B)** low initial input titer ($\sim 10^4$ TCID₅₀/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 cm² flasks at 2×10⁶
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 cm² 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 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 5×10^2 and 5×10^4 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 $20 \times 50\text{ cm}$
454 (VAH e.V.), precleaned with 70.0 % propan-1-ol or ethanol] were cut into pieces of $2 \times$
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 TCID₅₀ 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 μL 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 ~ 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 TCID₅₀/mL and 10^6 TCID₅₀/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 μ 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 μ 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 μ L EMEM (U373)
522 or DMEM (Vero E6) with trypsin. After 3 d or 6 d incubation at 37 °C in a CO₂-
523 atmosphere (5.0 % CO₂-content), cultures were observed for cytopathic effects.
524 TCID₅₀/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).
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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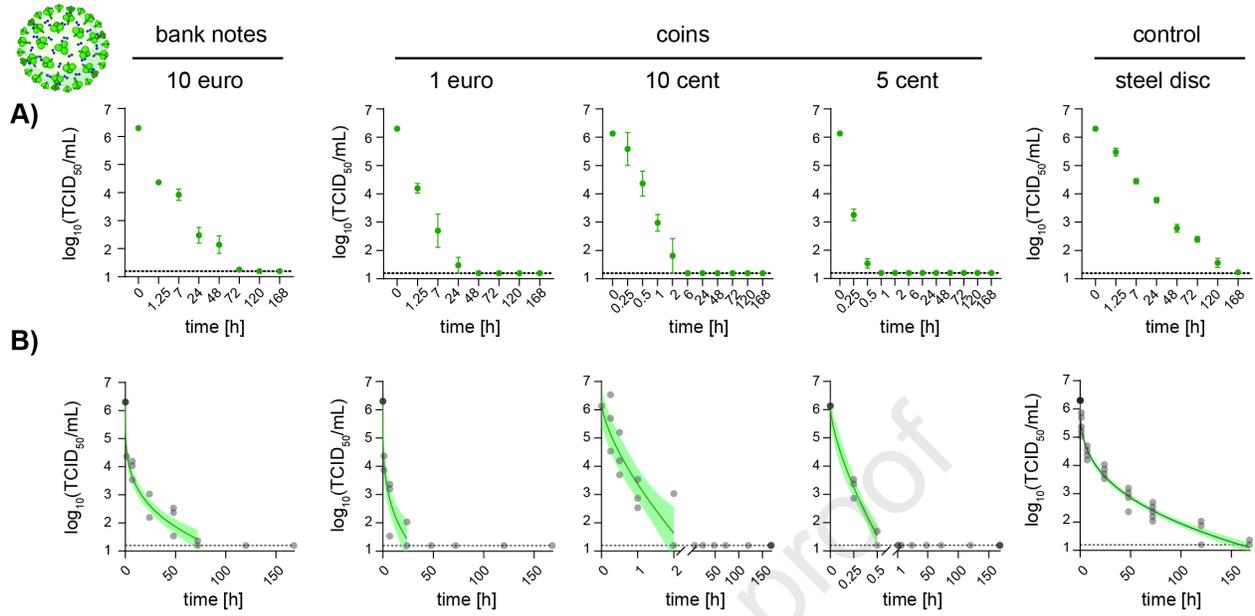
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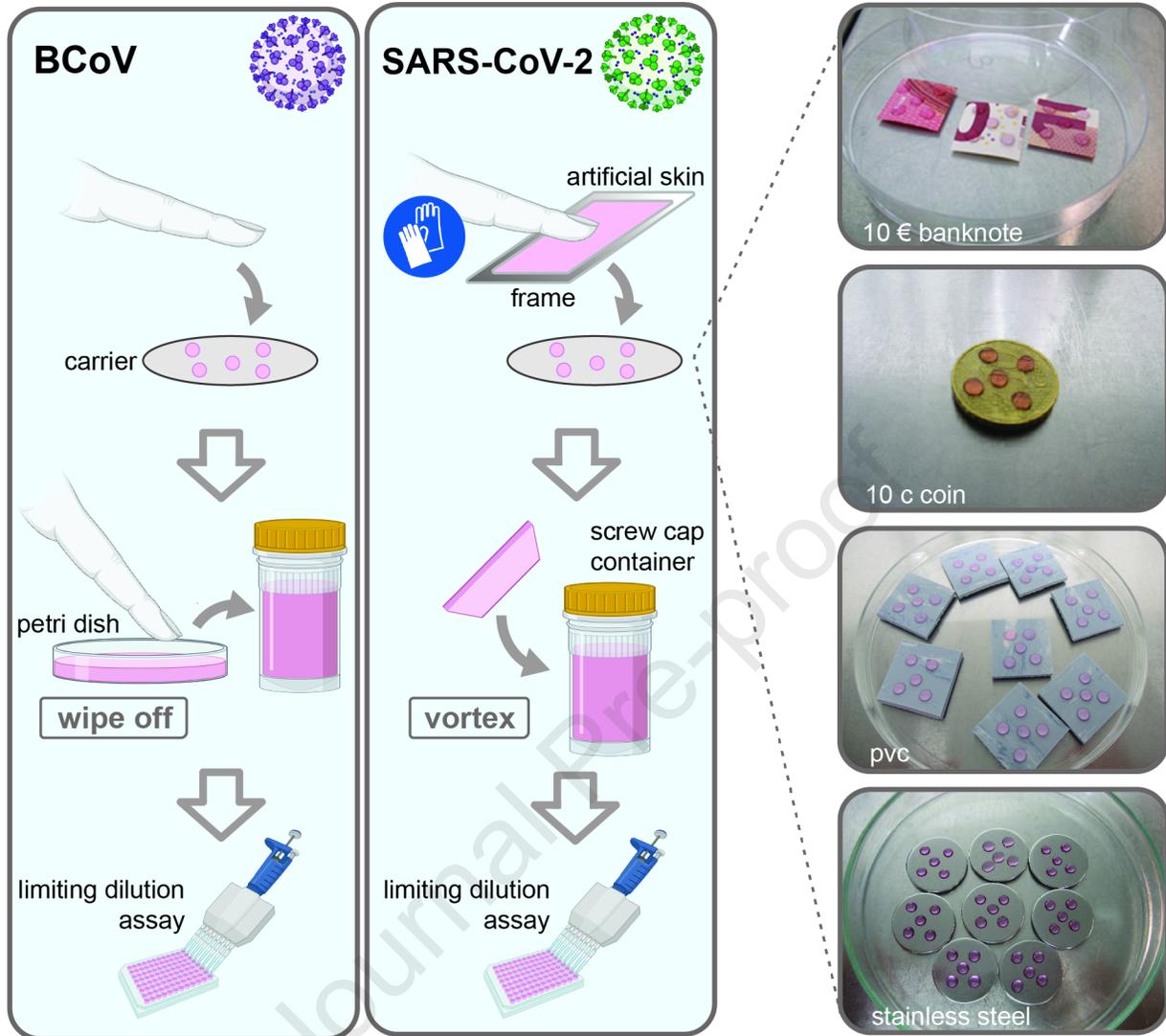
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Table 1: Initial decay time and time to reach lower limit of quantification (LLOQ) calculated from modelled curves.

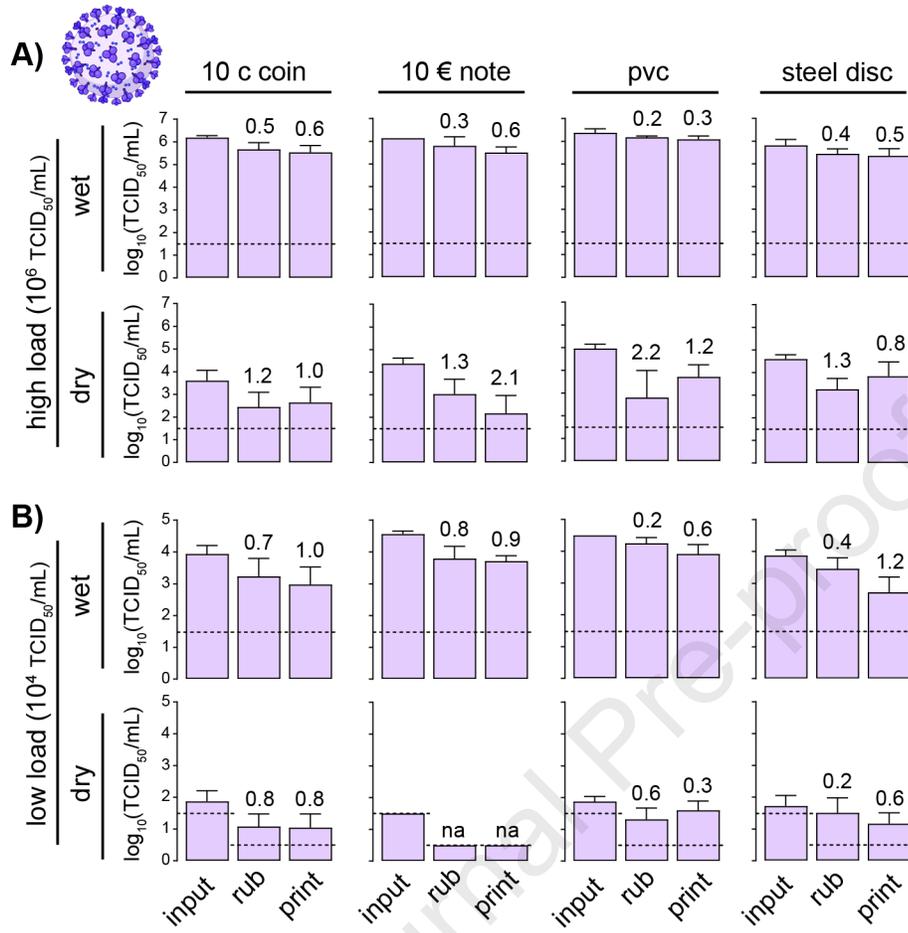
		SARS-CoV-2				BCoV	
		B.1.1.70 (wild type)		B.1.1.7 (alpha)			
	material	Initial decay [h]	time to LLOQ [h]	Initial decay [h]	time to LLOQ [h]	initial decay [h]	time to LLOQ [h]
notes	50 euro					2.8	175.6
	10 euro	6.1	85.7	0.22	59.23	6.5	216.3
coins	1 euro	2.2	28.4	0.74	70.72		
	10 cent	0.8	2.3	0.49	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

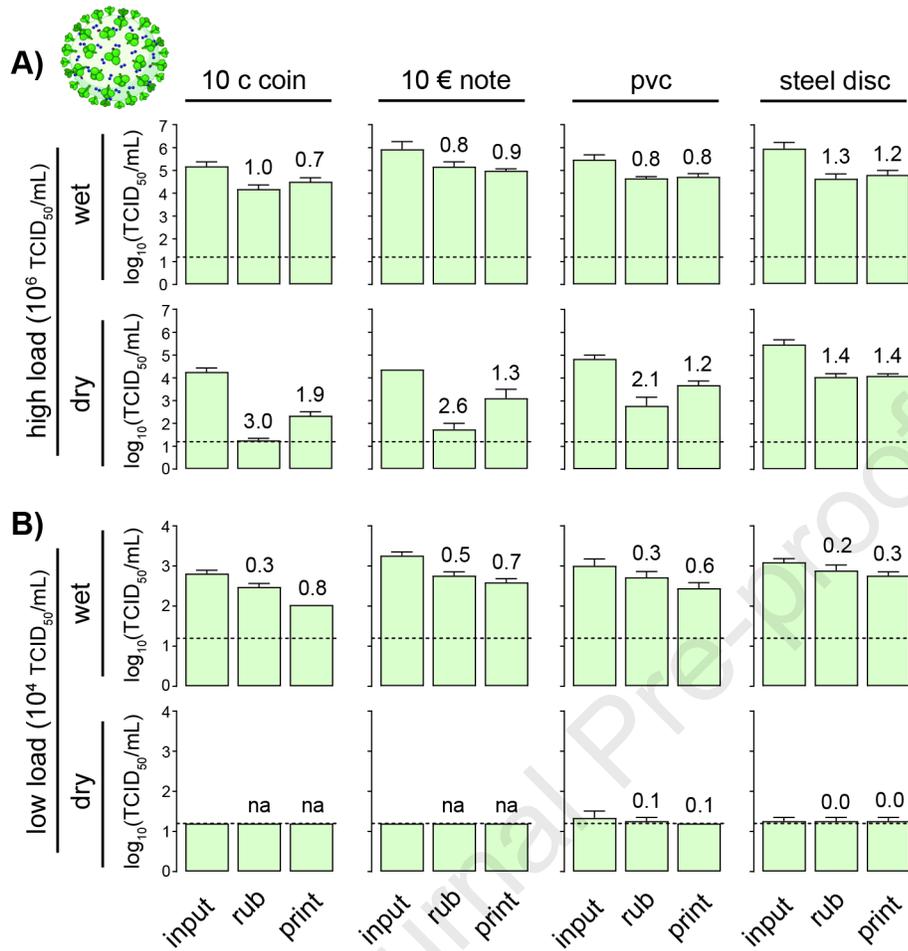


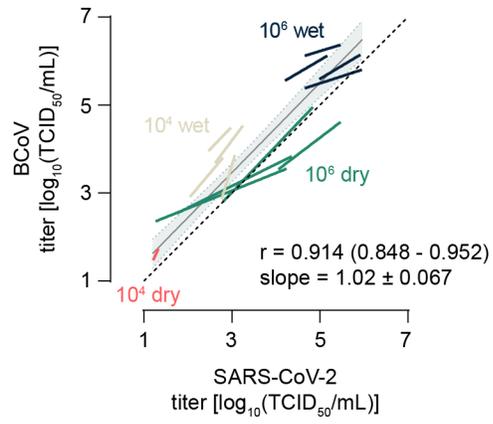


BCoV

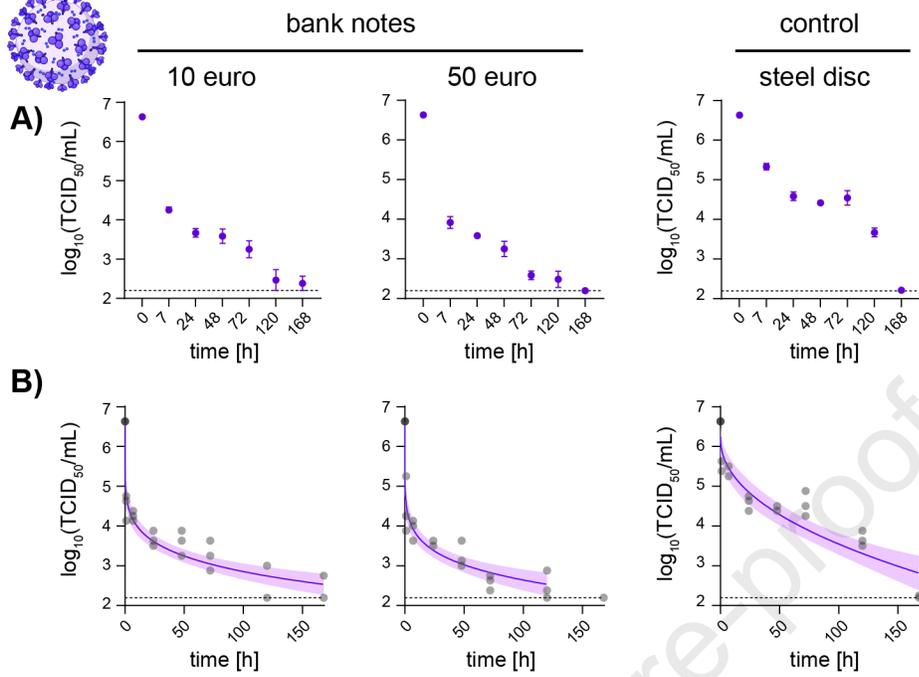
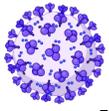


SARS-CoV-2





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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