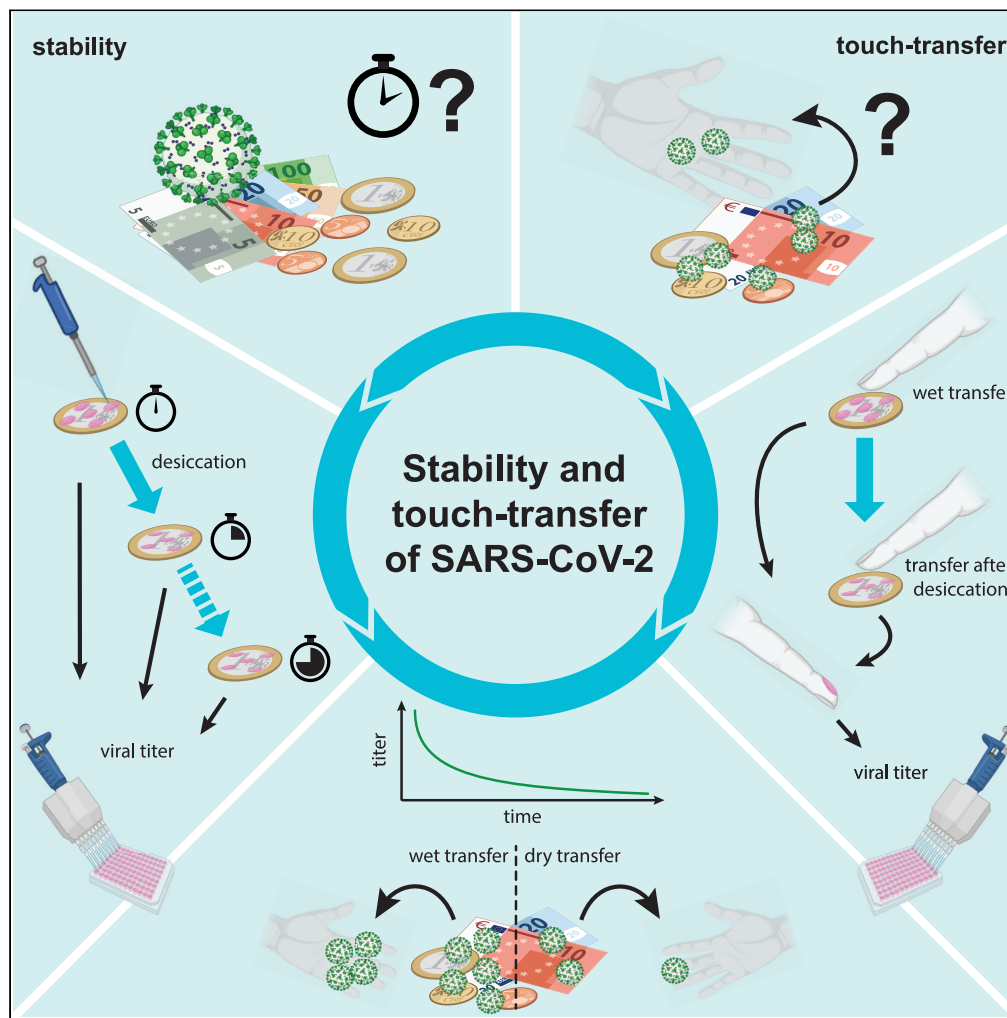


Article

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



Daniel Todt, Toni Luise Meister, Barbora Tamele, ..., Stephanie Pfaender, Yannick Brüggemann, Eike Steinmann

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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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Article

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

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SUMMARY

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

INTRODUCTION

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has created a significant threat to global health. Since effective treatments and access to vaccines is still limited for the broad population in most countries, diligent attention on transmission-based precautions is essential to limit viral spread. In particular, considering the emergence of novel SARS-CoV-2 variants displaying increased transmissibility, more severe disease and significant reduction in neutralization by antibodies can reduce the effectiveness of treatments or vaccines (Nicholas et al., 2021; Hou et al., 2020). According to current evidence, SARS-CoV-2 is mainly transmitted through respiratory droplets and aerosols exhaled from infected individuals (Kampf et al., 2020). Respiratory secretions or droplets expelled by infected individuals can potentially contaminate surfaces and objects (fomites) and have been shown to persist on inanimate surfaces for days under controlled laboratory conditions (Kratzel et al., 2020; van Doremalen et al., 2020). Therefore, a clinically significant risk of SARS-CoV-2 transmission by fomites has been assumed (Ong et al., 2020; Riddell et al., 2020; Chia et al., 2020). The COVID-19 pandemic intensified the decline in the transactional use of cash, partly due to reduced consumer spending but also due to concerns about the risk of banknotes transmitting the virus. This was observed for both sides, the retailers' and the customers (European Central Bank 2021). Indeed, frequently touched objects such as banknotes and coins have been suspected to serve as transmission vehicle of various pathogenic bacteria, parasites, fungi, and viruses including SARS-CoV-2 (Angelakis et al., 2014; Pal and Bhadada 2020). However, the conditions presented in various experimental studies often do not resemble real-life scenarios (e.g. large virus inoculums, small surface area) and thereby potentially exaggerate the risk of transmission of SARS-CoV-2 by fomites (Mondelli et al., 2020; Goldman 2020). Although different viruses are readily exchanged between skin and surfaces, the fraction of virus transferred is dependent on multiple factors including virus species and surface material (Julian et al., 2010). The efficiency of pathogen transfer from the fomite to hands is an important parameter to model its potential for transmission and to implement effective hygiene measures, while avoiding unnecessary measures (Lopez et al., 2013). However, the transfer of SARS-CoV-2 from surfaces to skin has not been analyzed systematically. Here, we examined the stability of SARS-CoV-2 and bovine coronavirus (BCoV) as surrogate on

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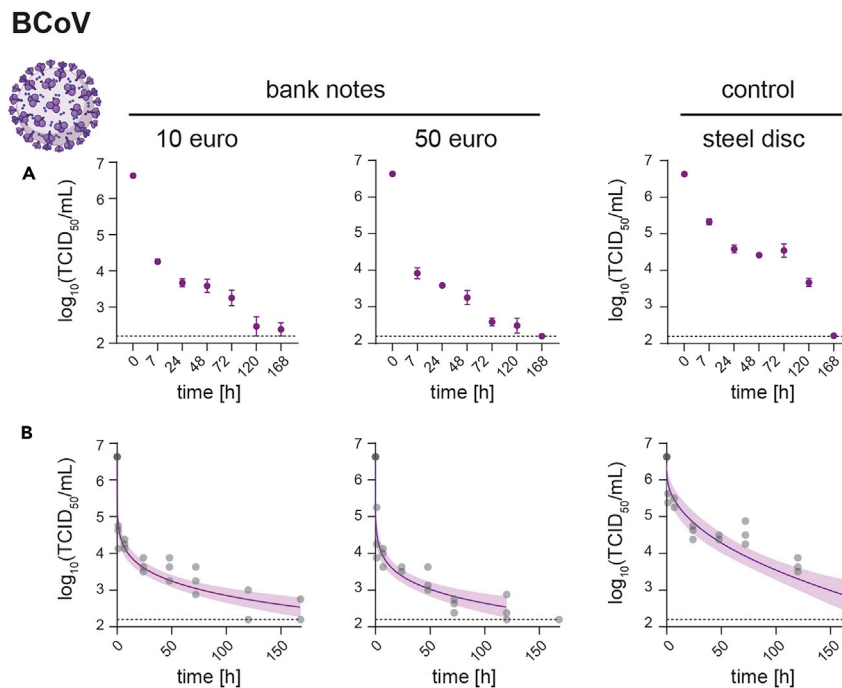


Figure 1. Stability of BCoV on banknotes and steel discs

BCoV stock solution was applied on 2 cm × 2 cm pieces of 10 € or 50 € banknotes and recovered after the indicated times. Residual titer was assessed via limiting dilution assay. Temperature during experiments was logged ($18^{\circ}\text{C} \pm 1^{\circ}\text{C}$ – $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

(A) Infectious BCoV recovered, displayed as raw $\text{TCID}_{50}/\text{mL}$ (y axes) over time (categorical x axes). Dots indicate mean values of three independent experiments with standard deviation, lower limit of quantification (LLOQ) is shown as dashed line.

(B) Recovered BCoV displayed as $\text{TCID}_{50}/\text{mL}$ (y axes) over time (continuous x axes). Dots represent individual biological experiments, purple lines and areas display the course of the Weibull distribution fitted data and 95% confidence interval, LLOQ is shown as dashed line. Virus particles created with [BioRender.com](https://www.biorender.com).

different means of payment. We further implemented a new protocol to study the touch transfer efficiency between fomites and fingertips. Importantly, we only observed a transfer between fomites and fingertips using a large initial virus titer sample (10^6 50% tissue culture infectious dose per milliliter ($\text{TCID}_{50}/\text{mL}$)) on the tested surfaces, while lower initial virus titer stocks (10^4 $\text{TCID}_{50}/\text{mL}$) were not effectively transferred.

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

RESULTS

Stability of BCoV on euro banknotes

To examine the stability of coronaviruses on banknotes, we first used BCoV, which can be cultivated under lower biosafety levels and has been used as a surrogate virus for inactivation studies replacing the highly pathogenic MERS-CoV and SARS-CoV ([Siddharta et al., 2017](#)). All euro banknotes are made of pure cotton fiber. To protect the surface of banknotes with smaller denomination and prolong circulation life, 5 € and 10 € banknotes are coated with a varnish applied after printing ([European Central Bank 2017](#)). To account for the effect of this varnish on surface stability of BCoV over time, we assessed residual infectivity from pieces of 10 € and 50 € banknotes for 7 h, 24 h and subsequently every 24–48 h up to 7 days ([Figure 1A](#)). The initial virus concentration of 4.3×10^6 $\text{TCID}_{50}/\text{mL}$ declined to 1.84×10^4 $\text{TCID}_{50}/\text{mL}$ on 10 € banknotes and 9.25×10^4 $\text{TCID}_{50}/\text{mL}$ on 50 € banknotes after 7 h desiccation. To quantitatively compare this early loss of titer on the different surfaces, we employed a fitted Weibull distribution model to estimate initial decay rates and the modeled time to (lower limit of quantification [LLOQ]) ([Figure 1B](#), [Table 1](#)). For both banknotes we observed shorter initial decay (2.75 h on 50 € and 6.45 h on 10 €) as compared with the steel disc (53.54 h) ([Figure 1B](#), [Table 1](#)). Following the strong initial decay, we were able to detect low amounts

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Table 1. Initial decay time and time to reach lower limit of quantification (LLOQ) calculated from modeled curves

		SARS-CoV-2					
		B.1.1.70 (wild-type)		B.1.1.7 (alpha)		BCoV	
	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					2.75	175.62
	10 euro	6.07	85.67	0.22	59.23	6.45	216.31
Coins	1 euro	2.21	28.43	0.74	70.72		
	10 cent	0.83	2.28	0.49	37.07		
	5 cent	0.20	0.55	0.12	2.25		
Control	Steel disc	20.59	158.83	1.21	882.92	53.54	240.17

of infectious virus after 120 h (50 €) and 168 h (10 €), respectively (Figure 1A), which is very much in line with the observed times in the model of 175.62 h for 50€ and 216.31 h for 10 € notes (Figure 1B and Table 1). In contrast, on steel discs a more continuous decay was observed and infectious virus could be recovered up to 120 h (Figure 1A), and 240.17 h for the fitted model, respectively (Figure 1B, Table 1).

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

We next examined the surface stability of infectious SARS-CoV-2 on 10 € banknotes, different coins (1 €, 10 cent, 5 cent) and stainless-steel discs for up to 7 days using an initial virus concentration of $1.36 - 2.0 \times 10^6$ TCID₅₀/mL (Figure 2). On 10 € banknotes and 1 € coins, the initial virus concentration declined to 2.32×10^4 TCID₅₀/mL and 1.79×10^4 TCID₅₀/mL, respectively, after 1.25 h, corresponding to an estimated initial decay time of 6.07 h and 2.21 h (Figure 2B, Table 1). No infectious virus could be recovered after 72 h and 48 h (Figure 2A) matching 85.67 h and 28.43 h survival time (Figure 2B, Table 1). In contrast, on 10 cent and 5 cent coins, the initial virus concentration declined to 5.96×10^4 TCID₅₀/mL and 3.86×10^1 TCID₅₀/mL, respectively, within 30 min. Initial decay rates were calculated as 49.8 min (10 cent) and 12 min (5 cent) (Figure 2B, Table 1). Importantly, from 10 cent coins no infectious virus could be recovered after 6 h, while for 5 cent coins infectivity was completely lost after 1 h (Figure 2A), as reflected by 2.28 h and 33 min survival time for SARS-CoV-2 on 10 cent and 5 cent coins (Figure 2B, Table 1). In contrast, on stainless-steel discs, which served as reference material, initial decay and time to reach background levels were comparable to BCoV with 20.59 h and 158.83 h, respectively (Figure 2B, Table 1). Virus titers declined more evenly until no infectious virus could be recovered after 120 h (Figure 2A). Likewise, we examined the surface stability of the SARS-CoV-2 alpha variant on 10 € banknotes, different coins (1 €, 10 cent, 5 cent) and stainless-steel discs for up to 7 days using an initial virus concentration of 6.87×10^6 TCID₅₀/mL (Figure S1). On all surfaces, the initial virus concentration declined to $\sim 5 - 1 \times 10^4$ TCID₅₀/mL after 0.25 h (Figure S1A). However, on 10 € banknotes, 1 € and 10 cent coins no infectious virus could be recovered after 7 h and 24 h, while on 5 cent coins the virus was already inactivated after 2 h. In contrast, on stainless-steel discs, following the initial decay, infectious virus could be recovered for up to 7 days. Quantitative estimates of the initial decay rates were comparable to wild-type SARS-CoV-2 (Figure S1B, Table 1). Time to reach LLOQ was decreased on 10 € banknotes, while on coins slightly longer times were modeled (Table 1). Overall, we observed comparable inactivation kinetics on the different materials for the SARS-CoV-2 alpha variant of concern when compared with the wild-type virus.

Development of a touch transfer assay to study virus transfer between cash and fingertips

Experiments performed under controlled laboratory conditions demonstrated the persistence of SARS-CoV-2 on inanimate surfaces for days and consequently implied the risk of viral transmission via contaminated objects (Chin et al., 2020; van Doremalen et al., 2020). However, to develop more refined models to assess the risk of fomites-based transmission of SARS-CoV-2, quantitative measurements of the transfer efficiency of infectious virus between skin and surfaces are required. To address these limitations, we developed a touch transfer assay to study the transfer of infectious BCoV and SARS-CoV-2 between fingertips and different fomites (Figure 3). Briefly, virus suspensions were placed on different surfaces (pieces of 10 € banknotes, 10 cent coins, pieces of PVC to mimic the surface of credit cards and stainless-steel discs as reference material). Afterwards, the wet inoculum or the dried suspension was touched by "printing" or "rubbing" using fingertips (BCoV) or an artificial skin fabric (SARS-CoV-2) (Figure 3). Subsequently,

SARS-CoV-2

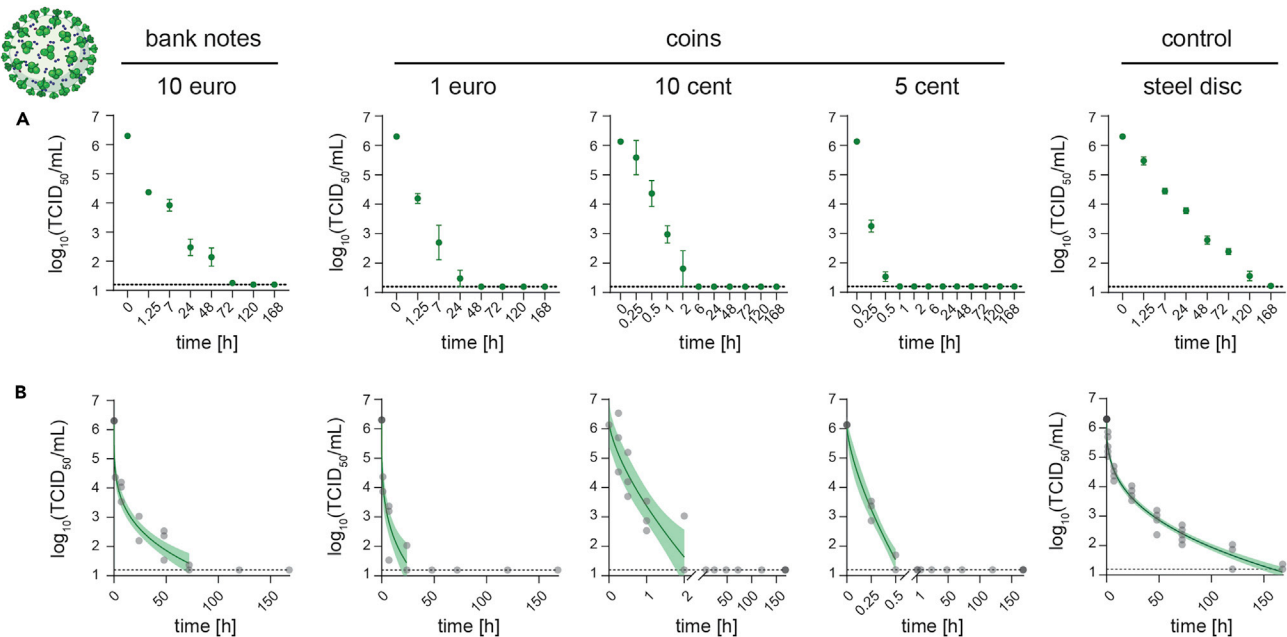


Figure 2. Stability of SARS-CoV-2 on banknotes, coins and steel discs

SARS-CoV-2 stock solution was applied on 2 cm × 2 cm pieces of 10 € banknotes, 1 €, 10 cent, and 1 cent coins and recovered after the indicated times. Residual titer was assessed via limiting dilution assay. Humidity and temperature during experiments was logged (32%–43% RH, 22.4°C–23.2°C)

(A) Infectious SARS-CoV-2 recovered, displayed as raw $\text{TCID}_{50}/\text{mL}$ (y axes) over time (categorical x axes). Dots indicate mean values of three independent experiments with standard deviation, lower limit of quantification (LLOQ) is shown as dashed line.

(B) Recovered SARS-CoV-2 displayed as $\text{TCID}_{50}/\text{mL}$ (y axes) over time (continuous x axes). Dots represent individual biological experiments, green lines and areas display the course of the Weibull distribution fitted data and 95% confidence interval, LLOQ is shown as dashed line. Virus particles created with [BioRender.com](https://www.biorender.com).

infectious viruses were recovered by dipping and rubbing each fingertip in turn for one minute on the base of a Petri dish containing 2 mL of EMEM cell culture medium (BCoV) or, in case of the artificial skin, by directly placing it into a container with cold DMEM (SARS-CoV-2). The resulting suspension was serially diluted to determine $\text{TCID}_{50}/\text{mL}$ values of the remaining infectious virus.

Transferability of BCoV from banknotes, coins and PVC to fingertips

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

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

Next, we examined the transmission of infectious SARS-CoV-2 from surfaces to fingertips. Surfaces were inoculated with either a high ($\sim 1 \times 10^6 \text{ TCID}_{50}/\text{mL}$) or low ($\sim 1 \times 10^4 \text{ TCID}_{50}/\text{mL}$) titer to represent different

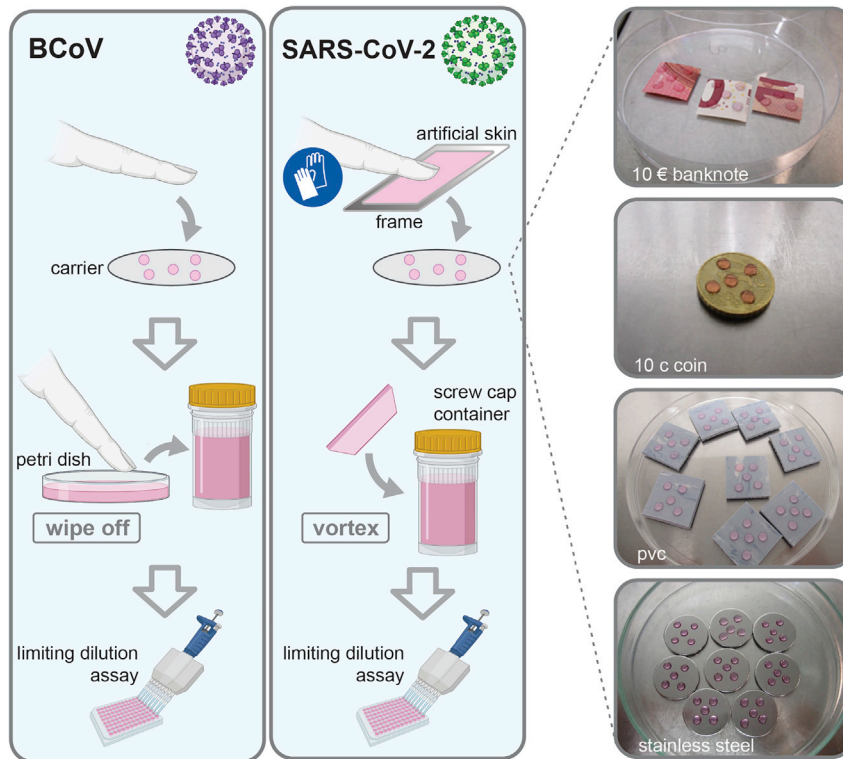


Figure 3. Touch transfer assay setup

To study the transfer of infectious BCoV and SARS-CoV2 between fingertips and different fomites, 50 μ L virus suspensions are placed on different surfaces (pieces of 10 € banknotes, 10 cent coins, pieces of PVC to mimic the surface of credit cards and stainless-steel discs as reference) in 10 μ L spots. Afterward, the wet inoculum or the dried suspension is touched by “printing” or “rubbing” using fingertips (BCoV) or an artificial skin fabric (SARS-CoV-2). Subsequently, infectious virus was recovered by rubbing the fingertip on the bottom of a Petri dish filled with respective culture media or in case of the artificial skin directly transferred into a container. The resulting suspension is serially diluted to determine TCID₅₀/mL values of the remaining infectious virus. Created with [BioRender.com](https://www.biorender.com).

degrees of surface contamination. As described before, virus transfer was assessed directly following inoculation (wet) or after drying either by printing (print) or rubbing (rub) the fingertip onto the surface. For a high initial titer and direct surface contact, we observed a maximum of a 1 log₁₀ reduction for the 10 cent coin, while lower reduction factors were observed for the other surfaces (Figure 5A). Drying of the initial inoculum led to ~1 log reduction in virus titer. In the dried state, less virus was transferred and could be recovered, e.g. by printing the fingertip we observed a 3.0 log₁₀ reduction on the 10 cent coin, while lower reduction factors were observed for the other surfaces. For a low initial titer and direct surface contact, we observed the highest reduction on the 10 € banknote (0.7 log₁₀ reduction). In case of drying the initial inoculum followed by a fingerprint we observed a reduction of the initial inoculum after 1 h desiccation to close/under the limit of quantification and only from the PVC very low (2.19×10^1 TCID₅₀/mL) amounts of infectious virus could be recovered (Figure 5B).

DISCUSSION

Human-to-human transmission of SARS-CoV-2 occurs primarily by respiratory aerosols or droplets and subsequent contact to nasal, oral, or ocular mucosal membranes. Based upon virus stability on surfaces, fomite transmission of SARS-CoV-2 has been considered possible (Chin et al., 2020; van Doremalen et al., 2020; Biryukov et al., 2020; Bueckert et al., 2020; Kwon et al., 2021; Riddell et al., 2020); however, the importance of this route in health care and public settings remains controversial (Goldman 2020; Mondelli et al., 2020; Pitol and Julian 2021). Fomite-based transmission has been proposed to contribute to the spread of other common respiratory pathogens (Kraay et al., 2018; Boone and Gerba 2007). Consequently, paper currency and coins have been suspected as a potential transmission vehicle for various pathogens, including

BCoV

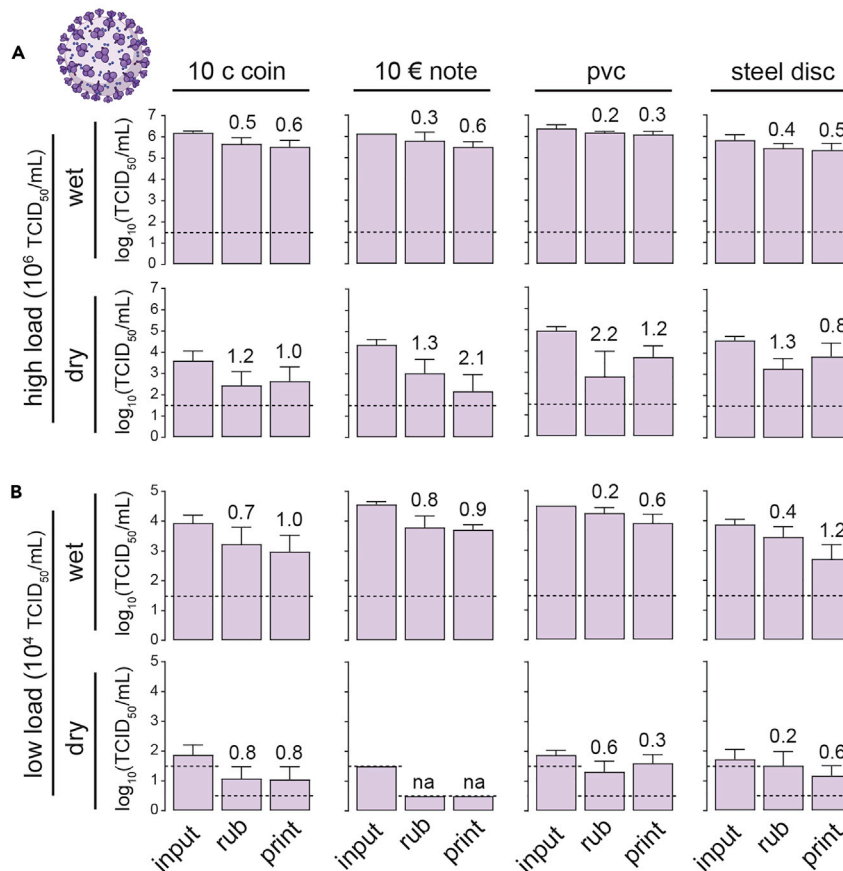


Figure 4. Transferability of BCoV from cash fomites to fingertips

Bars depict titer of input virus suspension and recovered infectious virus from different cash fomites, i.e. 10 cent coin, 10 € banknote, PVC and steel disc carrier in four different scenarios; mean \pm SD. Temperature during experiments was logged ($18^{\circ}\text{C} \pm 1^{\circ}\text{C}$ – $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

(A and B) (A) High initial input titer ($\sim 10^6$ TCID₅₀/mL) wet, when directly touch after application and dry, when transferred after visual desiccation and (B) low initial input titer ($\sim 10^4$ TCID₅₀/mL), wet and dry. Each scenario was performed by three test persons using eight fingers each. Numbers above bars indicate reduction factor, lower limit of quantification (LLOQ) is shown as dashed line. Virus particles created with [BioRender.com](https://www.biorender.com).

SARS-CoV-2 (Pal and Bhadada 2020; Angelakis et al., 2014; Xiao et al., 2017). Although infectious viruses have not been directly detected on banknotes or coins, the potential for their transmission has been proposed because of the observation that human influenza viruses were able to persist and remain infectious for several days when they were deposited on banknotes (Thomas et al., 2008). Furthermore, many other viruses, (i.e. Adenoviruses, Rotaviruses) are stable in the environment and exhibit high infectivity and, thus, could possibly be transferred by banknotes and coins (Wißmann et al., 2021). In agreement with previous reports, we found that high titers of SARS-CoV-2 and its surrogate BCoV, after an initial loss of infectivity, remained infectious for days under laboratory conditions on banknotes and coins (Table 1, Figures 1 and 2) (Harbourt et al., 2020; Chin et al., 2020). The initial loss of infectivity was higher on coins and banknotes, irrespective of protective varnish, when compared with stainless steel, indicating faster desiccation due to liquid absorption (banknotes) or antiviral surface properties (e.g. copper in coins). Both BCoV and SARS-CoV-2 displayed highly comparable levels of virus transfer and stability among the different conditions (Figure 6), implying that BCoV is also a suitable surrogate virus to model surface transmission of SARS-CoV-2.

Decay of SARS-CoV2 is likely determined by a combination of the initial amount of infectious virus deposited on a given surface and other environmental parameters (temperature, humidity, media components,

SARS-CoV-2

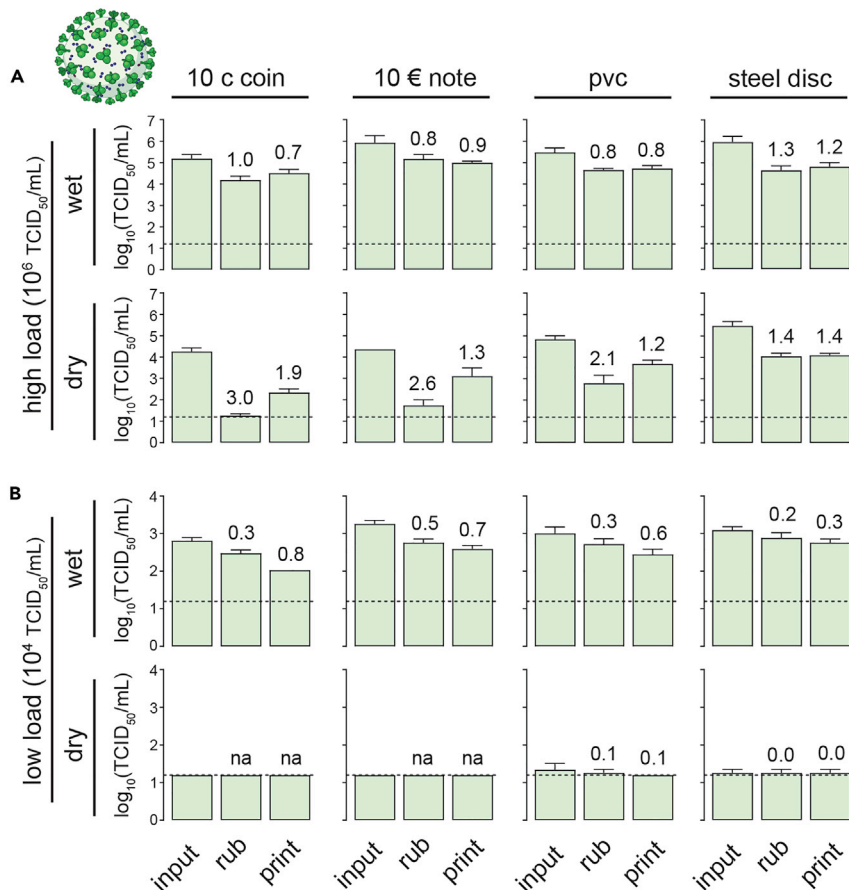


Figure 5. Transferability of SARS-CoV-2 from cash fomites to fingertips

Bars depict titer of input virus suspension and recovered infectious virus from different cash fomites, i.e. 10 cent coin, 10 € banknote, PVC and steel disc carrier in four different scenarios; mean \pm SD. Humidity and temperature during experiments was logged (32%–43% RH, 22.4°C–23.2°C) (A and B) (A) High initial input titer ($\sim 10^6$ TCID₅₀/mL) wet, when directly touch after application and dry, when transferred after visual desiccation and (B) low initial input titer ($\sim 10^4$ TCID₅₀/mL), wet and dry. Numbers above bars indicate reduction factor, lower limit of quantification (LLOQ) is shown as dashed line. Virus particles created with [BioRender.com](https://www.biorender.com).

light, and UV conditions). For example, the reduction of viral titers after drying was lower for the high viral load compared with the low viral load samples, indicating a dose-dependent effect of the viral decay after drying. Interestingly, this dose-dependency is in line with findings for survival of SARS-CoV-1 on paper and cotton (Lai et al., 2005). Furthermore, persistence of pathogens in the environment represents only the first requirement for self-inoculation via contaminated fingers. However, the possibility of fingerprint transmission has quantitatively been examined only in the context of bacteria (Knobloch et al., 2017; Chen et al., 2001). Using a newly developed virus touch transfer assay, we observed that the transfer of BCoV and SARS-CoV-2 between fomites and fingertips is context-dependent: For a high initial virus titer ($\sim 10^6$ TCID₅₀/mL), the transfer was more efficient for the wet inoculum, while visual desiccation on the one hand resulted in reduction of the titer as outlined above, as well as less efficient mobilization of the viral particles, reflected by higher reduction factors. Consequently, lower viral burdens ($\sim 10^4$ TCID₅₀/mL) mimicking more realistic real-life contamination events, as observed for influenza viruses in aerosol particles from human coughs (Lindsley et al., 2010; Goldman 2020), were not effectively transferred (Figures 4 and 5). Recent studies estimated a minimal infectious dose of SARS-CoV-2 in the range of 3×10^2 to 2×10^3 viral particles (Popa et al., 2020; Basu 2021), or as low as 100 particles (Karimzadeh et al., 2021). Overall, our results point to a low risk of SARS-CoV-2 transmission by coins and banknotes and the rush to abandon cash during the pandemic seems unnecessary.

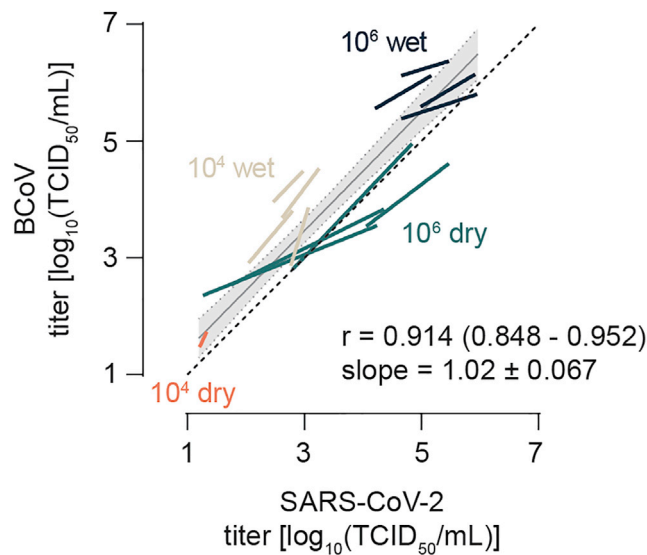


Figure 6. Suitability of BCov as surrogate for SARS-CoV-2 in touch transfer studies

Titers of recovered infectious virus were matched between BCov and SARS-CoV-2 for each scenario and linear regression curves calculated for input, rub and print. Gray line and area represent the overall linear regression and 95% confidence interval of all matched data points, dashed line depicts perfect correlation.

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

Limitations of the study

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

In a worst-case scenario including high virus loads (directly coughing or sneezing on the coin or banknote) and high transfer efficiencies (wet body liquid) with neglectable inactivation by desiccation (immediate money transfer), SARS-CoV-2 transmission might be possible. While our results clearly show that SARS-CoV-2 can be transferred from banknotes/coins to finger tips under certain conditions (large inoculum

and wet transfer), an additional step is required to transfer the virus to the respiratory system: transfer from fingertips to the nose or mouth and respective mucosal surfaces. A quantitative microbiological risk assessment of SARS-CoV-2 transmission in real life via banknotes/coins should consider these steps and relate the amount of remaining infectious virus to the human infectious dose.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2021.102908>.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.T., B.T., J.H., F.H.B., J.S.T., S.P., E.S; data curation, D.T., T.L.M., D.P., B.B., M.W., J.S; formal analysis, D.T. and J.S; funding acquisition, B.T., J.H., and E.S; investigation, T.L.M., D.P., B.B., N.H., V.K., and B.M.; methodology, D.T., F.H.B., S.P., and E.S; project administration, B.T, J.H., F.H.B., and E.S; resources, F.H.B., B.M., C.G., A.K., J.S.T., S.P., and E.S. validation, D.T., T.L.M., D.P., B.B., F.H.B., M.W., J.S., and E.S; visualization, D.T. and Y.B; writing – original draft, D.T., Y.B., S.P., and E.S; writing – review & editing, all authors.

DECLARATION OF INTERESTS

D.T. receives consulting fees from the European Central Bank. E.S. receives consulting fees from the European Central Bank and is a member of the scientific advisory board of Dr. Brill + Partner GmbH. F.H.B. is executive partner of Dr. Brill + Partner GmbH.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
hCoV-19/Germany/BY-Bochum-1/2020	In house	GISAID: EPI_ISL_1118929
RKI-0026_B.1.1.7	In house	GISAID: EPI_ISL_751799
BCoV strain L9	Dr. G. Zimmer, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, DE - 30559 Hannover)	NCBI: txid11130
Chemicals, peptides, and recombinant proteins		
Cristal violet	Merck	Cat No.: 1.01408
Dulbecco's modified Eagle's medium	Thermo Fisher	Cat No.: 11965092
Minimum Essential Medium Eagle	Biozym Scientific GmbH	Cat No.: 880120
Fetal Calf Serum	Thermo Fisher	Cat No.: 10270106
Fetal Calf Serum	Thermo Fisher	Cat No.: CH30160.02
non-essential amino acids	Thermo Fisher	Cat No.: 11140050
non-essential amino acids	Sigma-Aldrich-Chemie GmbH	Article No.: M7145
Penicillin/streptomycin	Thermo Fisher	Cat No.: 15070063
Penicillin/streptomycin	Sigma-Aldrich-Chemie GmbH	Article no. P-0781
L-Glutamine	Thermo Fisher	Cat No.: A2916801
L-Glutamine	Lonza	article no.: BE17-605E
amphotericin B	Thermo Fisher	Cat No.: 15290026
BSA Fraction V	AplliChem	Cat No.: A1391
BSA Fraction V	Sigma-Aldrich-Chemie GmbH	Cat No.: CA-2153
sodium pyruvate	Sigma-Aldrich-Chemie GmbH	Article no.: A S8636
phosphate buffered saline	Invitrogen	Article no.: 18912-014
Trypsin	Lonza	Article no.: BE17-161E
propan-1-ol	Merck KGaA	Article no.: 1.00996.1000
Ethanol	Carl Roth GmbH + Co. KG	9065.1
5 % (v/v) Decon 90®	Zinsser Analytic GmbH	Article no.: 80000
Experimental models: Cell lines		
Vero E6 cells	C. Drosten and M. Müller	NA
U373 cells	Dr. G. Zimmer, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, DE - 30559 Hannover)	NA
Software and algorithms		
GraphPad Prism version 9.1.1 for Windows	GraphPad Software	NA
Excel 2019	Microsoft Corporation	NA
Other		
Stainless steel discs	GF Formblech	10000-3021, 4174-3000
VITRO Skin® N-19 Starter Kit	IMS	NA

RESOURCE AVAILABILITY

Lead contact

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

Materials availability

This study did not generate new unique materials.

Data and code availability

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

METHOD DETAILS

Preparation of test virus suspension

For preparation of SARS-CoV-2 test virus suspension, Vero E6 cells (kindly provided by C. Drosten and M. Müller – Charité, Germany) were seeded in 75 cm² flasks at 2×10^6 cells in Dulbecco's Modified Eagle's Medium (DMEM, supplemented with 10 % (v/v) fetal calf serum (FCS), 1 % non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM L-Glutamine). The monolayer was either inoculated with hCoV-19/Germany/BY-Bochum-1/2020 (B.1.1.70) (GISAID accession ID: EPI_ISL_1118929), which was isolated during the first wave in Northern Europe and closely resembles the original Wuhan outbreak strain harboring a G614D mutation in the spike protein, or alpha variant (RKI-0026_B.1.1.7) (GISAID accession ID: EPI_ISL_751799). Strains were checked for lineage specific features as described in the supplement to [Meister et al., \(2021\)](#) ([Meister et al., 2021](#)). After 3 days and upon visible cytopathic effect the supernatant was harvested by centrifugation at 1,500 rpm for 5 min at room temperature, aliquoted and stored at -80°C until further usage.

For preparation of BCoV virus suspension, U373 cells were cultivated in a 75 cm² flask in Minimum Essential Medium Eagle (EMEM) supplemented with L-glutamine, non-essential amino acids, sodium pyruvate and 10 % FCS. Before virus infection, cells were washed two times with phosphate buffered saline (PBS), incubated for 3 h with serum-free EMEM and were washed once with EMEM supplemented with trypsin. For virus production, BCoV strain L9 (NCBI: txid11130) was added to the prepared monolayer. After an incubation period of 24 to 48 h cells were lysed by a rapid freeze/thaw cycle followed by a low speed centrifugation in order to sediment cell debris. After aliquoting of supernatant, test virus suspension was stored at -80°C. For assays, nine volumes of test virus suspension were mixed with one volume of interfering substance solution (final concentration of 0.3 g/L bovine serum albumin (BSA) in PBS) according to European Testing guideline ([EN 16777, 2019](#), section 5.2.2.8). The tests were performed with two different virus concentrations, i.e. a titer of approximately 10^4 50% tissue culture infectious dose per milliliter (TCID₅₀/mL) and a titer of 10^6 TCID₅₀/mL corresponding to an absolute viral load of approximately 5×10^2 and 5×10^4 TCID₅₀, respectively.

Preparation of specimens

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

Inactivation assays and controls

For stability testing, specimens were placed aseptically in a Petri dish and inoculated with 50 µL of the virus inoculum [5×10 µL drops, i.e. four in every corner and one in the middle of the square]. After visible drying of the inoculum, the petri dishes were closed and the specimens were incubated until the end of the

appropriate exposure time (up to 7 days). All experiments were performed at room temperature ($18^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and a relative humidity in the range of 30-45%. After the respective time, the specimens were transferred to 2 mL cell culture medium (without FCS) in a 25 mL container and vortexed for 60 seconds to resuspend the virus. Directly after elution, series of ten-fold dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on cell culture. Final concentrations of interfering substances when applied to cells in first wells of TCID₅₀ assay was 7.5 mg/L BSA and 0.225% FCS.

Fifteen and 30 minutes, 1, 2, 7, and 24 hours and 2, 3, 5 and 7 days were chosen as application times. Eluates were retained after appropriate drying times and residual infectivity was determined.

The initial virus titer was determined by addition of 50 μL of the virus inoculum directly to 2 mL cell culture medium without any desiccation.

Touch transfer test

For the touch transfer test with BCoV, three test persons simulated the transfer by pressing a finger shortly on the dried inoculum on the respective carriers followed by rubbing once with pressure over the carrier. Virus transfer was either assessed directly following application to fomites (wet) or after ~ 1 h until desiccation time (dry). Three other test persons simulated the transfer by a fingerprint of 5 seconds on the dried inoculum on the different carriers. Each test person performed the transfer test separately with the two different virus concentrations (10^4 TCID₅₀/mL and 10^6 TCID₅₀/mL) with 8 fingers each. For each test person and virus concentration, two fingers were used for virus transfer without drying of the inoculum. The transfer procedure was the same as with the dried inoculum, i.e. after visual desiccation.

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

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

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

QUANTIFICATION AND STATISTICAL ANALYSIS

Determination of infectivity

Infectivity was determined by means of end point dilution titration using the microtiter process. For this, samples were immediately diluted at the end of the exposure time with ice-cold EMEM containing trypsin and 100 μL of each dilution were placed in 6 or 8 wells of a sterile polystyrene flat-bottomed plate with a preformed U373 (BCoV) or Vero E6 (SARS-CoV-2) monolayer. Before addition of virus, cells were washed twice with EMEM (U373) or DMEM (Vero E6) and incubated for 3 h with 100 μL EMEM (U373) or DMEM (Vero E6) with trypsin. After 3 d or 6 d incubation at 37°C in a CO₂-atmosphere (5.0 % CO₂-content), cultures were observed for cytopathic effects. TCID₅₀/mL was calculated according to the method of Spearman and Kärber (Wulff et al., 2012). Lower limit of quantification (LLOQ) was defined as theoretical titer which yields in all wells for the lowest virus dilution being positive, while all others are negative (prerequisite for reliable application of method of Spearman is all wells should be positive at least for the lowest virus dilution) (Vieyres and Pietschmann 2013).



Fitting of virus titer decay

To account for different virus decay during desiccation and under wet incubation conditions, we implemented a Weibull distribution fit in GraphPad Prism version 9.0.2 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Only time points with residual viral titers of at least one replicate above the LLOQ were used for modeling. We used stock virus titers as initial titers for modeling to account for rapid loss due to inactivation/desiccation. The initial decay represents the time until initial log(viral titers) decreased to half-maximal range. Time to LLOQ describes the crossing point of the modelled curves with the LLOQ.

Calculation of the reduction factor

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