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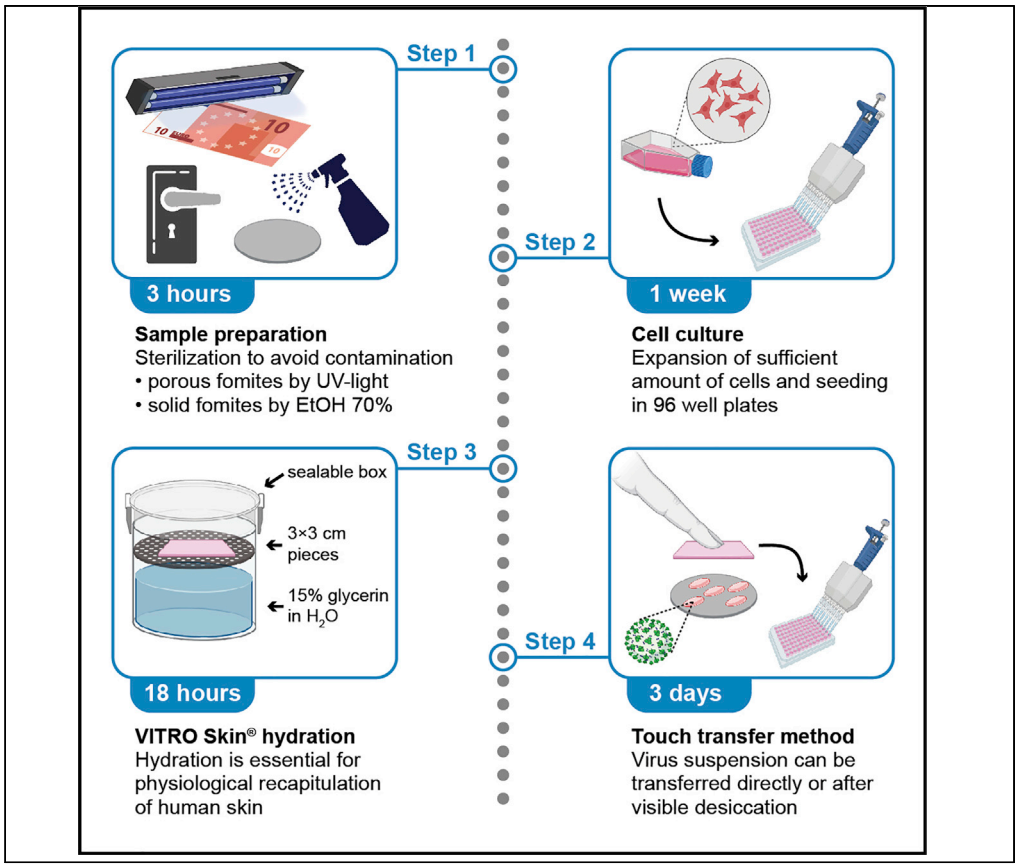
Protocol

A touch transfer assay to determine surface transmission of highly pathogenic viruses



Transmission via fomites poses a major dissemination route for many human pathogens, particularly because of transfer via fingertips. Here, we present a protocol to investigate direct transfer of infectious agents from fomites to humans via naked fingertips. The protocol is suitable for pathogens requiring highest biosafety levels (e.g., SARS-CoV-2). We used an artificial skin to touch a defined volume of virus suspension and subsequent quantification of infectious entities allows quantitative measurement of transfer efficiency and risk assessment.

Toni Luise Meister, Yannick Brüggemann, Barbora Tamele, John Howes, Eike Steinmann, Daniel Todt

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Highlights

A protocol to examine direct transfer of infectious agents from fomites to human skin

Suitable for pathogens requiring highest biosafety levels (e.g., SARS-CoV-2)

Artificial skin is used to touch defined amounts of investigated agents

Transfer efficiency is assessed by quantification of infectious entities

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A touch transfer assay to determine surface transmission of highly pathogenic viruses

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SUMMARY

Transmission via fomites poses a major dissemination route for many human pathogens, particularly because of transfer via fingertips. Here, we present a protocol to investigate direct transfer of infectious agents from fomites to humans via naked fingertips. The protocol is suitable for pathogens requiring highest biosafety levels (e.g., SARS-CoV-2). We used an artificial skin to touch a defined volume of virus suspension and subsequent quantification of infectious entities allows quantitative measurement of transfer efficiency and risk assessment.

For complete information on the generation and use of this manuscript, please refer to Todt et al. (2021).

BEFORE YOU BEGIN

Pathogen contaminants can be transmitted directly (i.e., respiratory droplets, hand-to-hand contact) or indirectly via contaminated surfaces (fomites) (Leung, 2021). In particular, frequently touched objects can serve as vehicles for transferring different clinically relevant pathogens. Banknotes and coins offer sample surface area and are frequently exchanged between individuals. As a result, numerous concerns have been raised, that banknotes and coins serve as vectors for the transmission of disease-causing microorganisms. During the recent COVID-19 pandemic, it was suspected that contaminated banknotes and coins contributed to the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting in a sharp decline in the use of cash (Tamele et al., 2021).

Although under controlled laboratory conditions, most pathogens are able to survive on different surfaces for a certain time frame (Wißmann et al., 2021), these measurements do not per se imply that a given surface can act as source of pathogen transmission if no disinfection is performed. In order to adequately assess the risk of transmission for a given pathogen and determine appropriate hygiene measures, detailed information about its survival on different surfaces and its ability to spread via contact with other surfaces is needed. In addition, the transfer efficiency is further determined by a combination of different parameters, including viral loads, virus species, and fomite material, requiring comprehensive measurements. However, for pathogens requiring high levels of biosafety (e.g., SARS-CoV-2) and permitting certain measurements (e.g., direct transfer of infectious agents from one specimen to another), comprehensive measurements and experimental settings similar to real-life conditions are notoriously difficult to achieve.

We recently implemented a new study protocol to quantitatively assess the transfer of SARS-CoV-2 between fomites and fingertips (Todt et al., 2021). To simulate the transfer of SARS-CoV-2 and

different means of payment, we inoculated banknotes and coins as well as stainless steel carriers with SARS-CoV-2 and recovered infectious virus by printing or rubbing an artificial skin fabric over the sample surface. The amount of recovered infectious virus was subsequently determined by an end-point dilution assay given in 50% tissue culture infectious dose per milliliter ($TCID_{50}$/mL). Based on different surfaces, initial virus titers, and application to fomites (wet or dry inoculum), our findings suggest a relatively low risk of SARS-CoV-2 transmission by contaminated coins and banknotes (Todt et al., 2021).

While our protocol, as described below, was initially developed to test whether SARS-CoV-2 can be transmitted from banknotes and coins to fingertips, it can easily be adapted to other highly pathogenic viruses under BSL3 biosafety precautions and/or scenarios of transmission.

SARS-CoV-2 propagation

Timing: 9 days

1. Prepare Dulbecco's Modified Eagle's Medium (DMEM complete; supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) non-essential amino acids, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM L-Glutamine)
2. Expand a sufficient amount of VeroE6 cells
   a. Seed VeroE6 cells at 2 × 10^6 cells per 75 cm^2 cell culture flask in 10 mL DMEM complete
   b. Incubate overnight at 37°C and 5% CO_2
3. Change medium to 10 mL fresh DMEM complete
   a. Inoculate monolayer with 100 µL of SARS-CoV-2 stock (virus titers should exceed 1 × 10^5 $TCID_{50}$/mL)
   b. Incubate 3 days and until visible cytopathic effect (CPE) at 37°C and 5% CO_2 (80%–100% of cells detached)
4. Harvest supernatant and subsequently centrifuge at 200 × g for 5 min at 18°C–25°C to remove any cell debris
   a. Collect supernatant, aliquot and store at −80°C until further usage
5. To determine infectious viral titers, seed cells at 1 × 10^4 cells in 200 µL DMEM complete per well in a 96 well plate
   a. Incubate overnight at 37°C and 5% CO_2
   b. Serially titrate the collected supernatant by adding 22 µL of the virus stock to the first row (sextuplicate), followed by thorough mixing and transferring 22 µL to the next row
   c. Continue serial dilution s for the remaining 6 rows.
   d. Incubate 3 days at 37°C and 5% CO_2
6. Discard the supernatant of the 96 well plate and add Crystal violet solution to each well
   a. Incubate 10 min at 18°C–25°C
   b. Discard Crystal violet and eventually wash the cells once with 1× phosphate buffered saline (PBS)
   c. Count wells that are positive for CPE and calculate the 50% tissue culture infectious dose ($TCID_{50}$) (see quantification and statistical analysis)

Pause point: Long-term storage of SARS-CoV-2 at −80°C.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
SARS-CoV-2 (hCoV-19/Germany/BY-Bochum-1/2020)	In house	GISAID: EPI_ISL_1118929

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Crystal Violet	Sigma-Aldrich	CAT#1.01408
Methanol	Carl Roth GmbH + Co. KG	CAT#X948.2
Ethanol	Carl Roth GmbH + Co. KG	CAT#9065.1
Dulbecco's modified Eagle's medium	Thermo Fisher Scientific	CAT#11965092
Penicillin/streptomycin	Thermo Fisher Scientific	CAT#15070063
L-Glutamine	Thermo Fisher Scientific	CAT#A2916801
Non-essential amino acids	Thermo Fisher Scientific	CAT#11140050
Fetal calf serum	Thermo Fisher Scientific	CAT#10270106
Amphotericin B	Thermo Fisher Scientific	CAT#15290026
Bovine serum albumin, fraction V	Sigma-Aldrich	CAT#CA-2153
Experimental models: Cell lines		
VeroE6	C. Drosten und M. Müller	N/A
Software and algorithms		
GraphPad Prism version 9.1.1 for Windows	GraphPad Software	N/A
Excel 2019	Microsoft Corporation	N/A
Other		
Stainless steel carrier	GK-Formblech	CAT#10000-3021
25 mL Container	Sarstedt	CAT#60.9922.115
VITRO Skin® N-19 Starter Kit (critical reagent)	IMS	N/A

STEP-BY-STEP METHOD DETAILS

Sample preparation

Timing: 3 h

Thorough preparation of fomites to be tested is essential to rule out misinterpretation of resulting data due to insufficient sterilization and ensure reproducibility.

1. Add 2 mL DMEM complete without FCS to each container (Sarstedt) and store at 4°C until further usage (chose dimension of container to ensure complete coverage of tested fomite with liquid)
   a. Each replicate requires one container
2. Solid fomites, such as stainless-steel carriers or coins, are sterilized in 70% Ethanol for 20 min and aseptically placed in a petri dish for drying
   a. Each fomite is tested in triplicates
3. Porous fomites, such as banknotes or PVC plates, were cut in 2 × 2 cm pieces and placed in a petri dish and UV-irradiated for 20 min from both sides
   a. Each fomite is tested in triplicates

⚠ CRITICAL: insufficient sterilization of the fomites can cause bacterial or fungal contamination during the experiment.

Pause point: Long-term storage of sterilized fomites under sterile conditions possible at 18°C–25°C. After four weeks we recommend anew sterilization.

Cell culture

Timing: up to one week (depending on the sample size)

Target cells are essential for the determination of residual infectivity after transfer. For SARS-CoV-2, VeroE6 cells are commonly used, but the protocol can be adjusted to respective virus and target cells.

4. Expand a sufficient amount of VeroE6 cells
   a. Each replicate requires half a 96 well plate for titration (sextuplicate)
5. Seed cells at 1 × 10^4 cells in 200 µL DMEM complete per well in a 96 well plate
   a. Incubate overnight at 37°C and 5% CO_2

VITRO Skin® hydration

Timing: 18 h

Hydration of the VITRO Skin® is essential for physiological recapitulation of human skin (https://ims-usa.com/vitro-skin-substrates/vitro-skin/).

6. Cut VITRO Skin® into pieces (3 cm × 3 cm) and place on sample holder
7. Add 15% (w/w) Glycerin in water to a big plastic box and place VITRO Skin® inside
8. Close lit and hydrate VITRO Skin® 16–18 h

Note: Step 6–8 of the step-by-step method details should be carried out at the same day as step 5. Once the VITRO Skin® is hydrated the experiment has to be carried out, as the artificial skin quickly dehydrates again. Thus, the skin should be kept under humid conditions throughout the whole experiment, e.g., by storing in a closed petri dish with a wet tissue inside.

Preparation of test virus suspension on fomites

Timing: 20 min

9. Nine volumes of SARS-CoV-2 are mixed with one-part interfering substance (3 g/L bovine serum albumin [BSA] in 1×PBS; final concentration in assay 0.3 g/L BSA in PBS) according to European Testing guideline (EN 16777, 2019, section 5.2.2.8)
   a. Tests were performed at two different virus concentrations (1 × 10^4 and 1 × 10^6 $TCID_{50}$/mL)
10. Five drops of 10 µL test virus suspension are spotted on each fomite (Figure 1A)
11. Depending on the scientific question, the contaminated fomites can be dried or used in a wet state before proceeding with the next step.

Note: Start with those fomites designated for drying the test virus suspension, as this process may take up to 1 h.

Touch transfer method

12. Fix the hydrated VITRO Skin® in the provided gray frame (Figure 1B)
    a. Rubbing procedure:
       i Put the index finger on the unruffled site of the VITRO Skin® and rub once across the fomite applying light pressure (Figure 1C)
       ii Proceed with step 13 of the step-by-step method details
    b. Print procedure:
       i Put the index finger on the unruffled site of the VITRO Skin® and lightly press the finger on the fomite for 5 s (Figure 1C)
       ii Proceed with step 13 of the step-by-step method details

Note: To achieve comparable results, the applied pressure should always be the same.

13. Unhinge the VITRO Skin® from the frame and transfer into a container filled with 2 mL DMEM w/o FCS (from step 1 of the step-by-step method details) (Figure 1D)

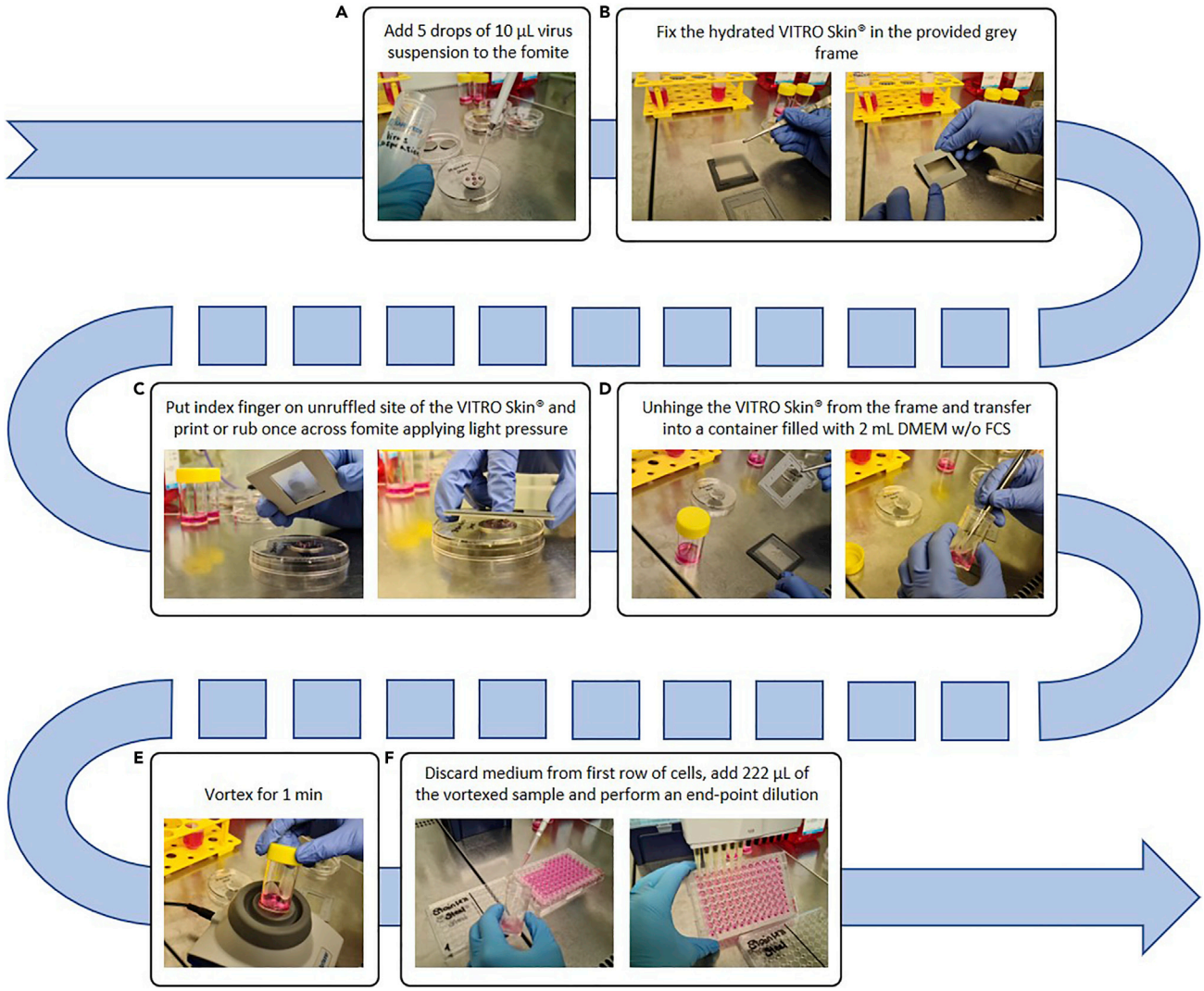

Figure 1. A method to determine transmission of SARS-CoV-2 via artificial skin in a BSL3 laboratory

(A) Each surface to be investigated is contaminated with 5 drops of 10 µL virus suspension. The virus suspension can either be directly subjected to the following steps or after desiccation.

(B) Hereafter, the hydrated VITRO Skin® is fixed in the provided gray frame.

(C) To recover residual infectious virus from the respective fomite, the index finger is put on the unruffled site of the VITRO Skin® and subsequently printed on the fomite for 5 s or rubbed across once.

(D and E) The VITRO Skin® is then unhinged from the frame, transferred into a container filled with 2 mL DMEM w/o FCS and vortexed for 1 min.

(F) To calculate the amount of infectious virus being recovered, an end-point-dilution assay is performed. Therefore, the medium from the first row of the seeded cells is discarded, 222 µL of the vortexed sample is added to each well and is serially dilute by thorough mixing and subsequently transferring 22 µL into the next row.

14. Vortex for 1 min (Figure 1E)
15. Discard cell culture medium from 6 wells of the first row of cells seeded in step 5 of the step-by-step method details and add 222 µL of the vortexed DMEM containing the VITRO Skin® (Figure 1F)
16. Serially dilute by thorough mixing and subsequently transferring 22 µL from the first row to the 200 µL in the second row (Figure 1F)
17. Repeat step 16 six times with subsequent rows
18. Incubate cells for 72 h at 37°C
19. Discard supernatant and add 200 µL crystal violet to each well

20. Incubate 10 min at 18°C–25°C
21. Discard supernatant and eventually wash with 1×PBS
22. Calculate residual viral titers ($TCID_{50}$/mL) (see quantification and statistical analysis)

Note: All experiments were performed at 18°C ± 1°C–25°C ± 1°C and a relative humidity in the range of 30%–45%.

⚠ CRITICAL: Depending on the research question and to simulate different transmission scenarios, step 11–22 can be carried out with desiccated virus or under wet conditions recapitulating different physical recovery methods of infectious virus. But, desiccation of the virus already leads to a reduction of viral titers. Therefore, high initial titers are required for this experimental setup to receive a sufficient measurement window.

EXPECTED OUTCOMES

Following the experimental setup described here, it is possible to assess SARS-CoV-2 transmission from different fomites to skin. However, this protocol can easily be adopted to other viruses by adjusting the cell line and/or read out or modified to study skin-to-skin transmission and other scenarios of transmission. Depending on initial viral titers a significant amount of virus can be transferred and subsequently recovered from the VITRO Skin®, which helps to calculate the risk of transmission by contact to contaminated surfaces. Indeed, frequently touched objects have been suspected to serve as vehicles for certain pathogens such as bacteria, parasites, fungi, and viruses including SARS-CoV-2 (Angelakis et al., 2014; Pal and Bhadada, 2020). Therefore, data providing information about transmission efficacy can play an important rule when adjusting hygiene measures.

TROUBLESHOOTING

Problem 1

After recovery of residual virus from the VITRO Skin®, no CPE can be observed on the target cells (step 22).

Potential solution 1

The lower limit of detection for end-point-dilution assays is higher compared to the more sensitive PCR, thus high viral loads of the initial virus stock are required to get a sufficient measurement window. When repeating the experiment, a virus stock with a higher titre should be used for the touch-transfer.

Problem 2

After recovery of residual virus from the VITRO Skin®, all wells in every dilution show CPE (step 22).

Potential solution 2

End-point dilution assays have distinct measurement windows, which may be exceeded when viral loads go beyond 10^8 $TCID_{50}$/mL. In this case the 2 mL DMEM w/o FCS from step 13 (Figure 1D) should be pre-diluted.

Problem 3

High variability between biological replicates is observed (step 22).

Potential solution 3

Viruses are sensitive to humidity and temperature. Make sure to conduct experiments under controlled environmental conditions to avoid increased/decreased desiccation and stability.

Problem 4

Dehydration of VITRO Skin® (step 8).

Potential solution 4

The hydrated VITRO Skin® should be stored in humid chambers to avoid dehydration. The best way to keep the skin hydrated is to add a wet tissue to the petri dish and only open the lid if necessary. Additionally, pay attention to not place the petri dish in the air flow of the work bench.

Problem 5

The VITRO Skin® disrupts when handled or hinged/unhinged from the frame (step 12 and 13).

Potential solution 5

The handling of the VITRO Skin® can sometimes be difficult, especially when hydrated as it becomes softer and at the same time more fragile. This can cause the artificial skin to disrupt. Therefore, users are advised to practice the handling of the skin and the frame.

QUANTIFICATION AND STATISTICAL ANALYSIS

Residual infectious viral titers were calculated according to Spearman and Kerber (Spearman, 1908; Kaerber, 1931). The lower limit of detection is set to 158 $TCID_{50}$/mL, given by the prerequisites for validity of this method, i.e., all wells are positive in the lowest dilution, while all other wells are negative (Vieyres and Pietschmann, 2013). A pre-filled Excel sheet for the $TCID_{50}$ calculation can be freely accessed at https://www.klinikum.uni-heidelberg.de/en/zentrum-fuer-infektiologie/molecular-virology/welcome/downloads (courtesy of Marco Binder, Heidelberg)

LIMITATIONS

Detection of residual viral titres should rely on the quantification of the infectious dose such as the calculation of the $TCID_{50}$, as the determination of viral loads by quantitative PCR does not provide information about infectiousness. However, the limit of detection for end-point-dilution assays is higher compared to the more sensitive PCR, thus high viral loads of the initial virus stock are required to get a sufficient measurement window.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Daniel Todt, Ruhr University Bochum, Germany (daniel.todt@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.

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AUTHOR CONTRIBUTIONS

T.L.M. conducted the experiments; T.L.M., B.T., J.H., E.S., and D.T. designed the experimental setup; T.L.M. and D.T. generated the figure; T.L.M., Y.B., and D.T. wrote the original manuscript draft; all authors reviewed the manuscript.

DECLARATION OF INTERESTS

D.T. and E.S. receive consulting fees from the European Central Bank. B.T. and J.H. are employees at the European Central Bank.

REFERENCES

Angelakis, E., Azhar, E.I., Bibi, F., Yasir, M., Al-Ghamdi, A.K., Ashshi, A.M., et al. (2014). Paper money and coins as potential vectors of transmissible disease. Future Microbiol. *9*, 249–261. https://doi.org/10.2217/fmb.13.161.

Kaerber, G. (1931). Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Pathol. Pharmakol. *162*, 480–482.

Leung, N.H.L. (2021). Transmissibility and transmission of respiratory viruses. Nat. Rev. Microbiol. *19*, 528–545. https://doi.org/10.1038/s41579-021-00535-6.

Pal, R., and Bhadada, S.K. (2020). Cash, currency and COVID-19. Postgrad. Med. J. *96*, 427–428. https://doi.org/10.1136/postgradmedj-2020-138006.

Spearman, C. (1908). The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae. Br. J. Psychol. *2*, 227–242.

Tamele, B., Zamora-Pérez, A., Litardi, C., Howes, J., Steinmann, E., and Todt, D. (2021). Catch Me (If You Can): Assessing the Risk of SARS-CoV-2 Transmission via Euro Cash. Occasional Paper Series. https://doi.org/10.2866/141321.

Todt, D., Meister, T.L., Tamele, B., Howes, J., Paulmann, D., Becker, B., Brill, F.H., Wind, M., Schijven, J., Heinen, N., et al. (2021). A realistic transfer method reveals low risk of SARS-CoV-2 transmission via contaminated euro coins and banknotes. IScience *24*, 102908. https://doi.org/10.1016/j.isci.2021.102908.

Vieyres, G., and Pietschmann, T. (2013). Entry and replication of recombinant hepatitis C viruses in cell culture. Methods (San Diego, Calif.) *59*, 233–248. https://doi.org/10.1016/j.ymeth.2012.09.005.

Wißmann, J.E., Kirchhoff, L., Brüggemann, Y., Todt, D., Steinmann, J., and Steinmann, E. (2021). Persistence of pathogens on inanimate surfaces: a narrative review. Microorganisms *9*, 343. https://doi.org/10.3390/microorganisms9020343.