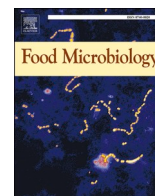




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Coronaviruses are stable on glass, but are eliminated by manual dishwashing procedures

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ABSTRACT

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is primarily transmitted from human to human via droplets and aerosols. While transmission via contaminated surfaces is also considered possible, the overall risk of this transmission route is assumed to be low. Nevertheless, transmission through contaminated drinking glasses may pose an increased risk as the glass is in direct contact with the mouth and oral cavity. Using human coronavirus 229E (HCoV-229E) as surrogate for SARS-CoV-2, this study examined coronavirus stability on glass, inactivation by dishwashing detergents, and virus elimination by a manual glass scrubbing device. Infectious HCoV-229E was recovered from glass for 7 and 21 days of storage under daylight and dark conditions, respectively. Near complete inactivation of HCoV-229E ($>4 \log_{10}$ reduction) was observed after incubation with two common dishwashing detergents at room temperature for 15 s, whereas incubation at 43 °C for 60 s was necessary for a third detergent to achieve a similar titer reduction. The virus was efficiently removed from contaminated drinking glasses using a manual glass scrubbing device in accordance with German standard DIN 6653-3. The results confirm that coronaviruses are relatively stable on glass, but indicate that common manual dishwashing procedures can efficiently eliminate coronaviruses from drinking glasses.

1. Introduction

The *Orthocoronavirinae*, a subfamily of the *Coronaviridae* family of the order *Nidovirales*, comprises a large group of coronaviruses, which represent enveloped viruses with a large single-stranded RNA genome (Walker et al., 2020). Within this subfamily, the human coronaviruses (HCoVs) belong to the genera *Alphacoronavirus* and *Betacoronavirus*. The two *Alphacoronavirus* members HCoV-229E (Tyrrell and Bynoe, 1965) and HCoV-NL-63 (van der Hoek et al., 2004) as well as the two *Betacoronavirus* members HCoV-OC43 (Hamre and Procknow, 1966) and HCoV-HKU-1 (Woo et al., 2005) are globally endemic and mostly cause mild respiratory symptoms of the common cold. Additional members of the *Betacoronavirus* genus, namely severe acute respiratory syndrome coronavirus (SARS-CoV) (Drosten et al., 2003; Ksiazek et al., 2003), Middle East respiratory syndrome coronavirus (MERS-CoV) (Zaki et al., 2012) and SARS-CoV-2 (Zhu et al., 2020), cause severe respiratory diseases. SARS-CoV and MERS-CoV are associated with high mortality rates of approximately 10% and 35%, respectively (WHO, 2015, 2019). However, outbreaks thus far remained confined and mostly limited to

healthcare-associated and zoonotic transmissions for SARS-CoV and MERS-CoV, respectively. In contrast, the initial estimated mortality rate of 0.66% for SARS-CoV-2 (Verity et al., 2020) is lower compared to SARS-CoV and MERS-CoV; however, SARS-CoV-2 is highly transmissible (Petersen et al., 2020). It is the causative agent of the ongoing coronavirus disease 2019 (Covid-19) pandemic (WHO, 2020), with almost 500 million cumulative cases and over 6 million deaths globally as of March 20th 2022 (WHO, 2022).

The predominant transmission route of SARS-CoV-2 is from human to human through respiratory droplets and aerosols and the virus remains infectious in aerosols for hours (van Doremalen et al., 2020; Zhang et al., 2020). However, fomite transmission was also considered possible, as the virus can remain infectious on surfaces for several days (van Doremalen et al., 2020) and has been recovered from household surfaces of SARS-CoV-2-infected individuals (Marcenac et al., 2021). The stability of HCoV on a variety of surfaces depends on the inoculation medium, inoculation substrate, relative humidity, UV exposure, virus titer, and temperature. HCoV stability on surfaces ranges from several hours to several days and has recently been extensively reviewed

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(Bueckert et al., 2020). The longest time-period after which infectious HCoV was still extracted from a surface was 28 days in the dark at 20 °C from various non-porous surfaces including glass (Riddell et al., 2020).

An oral transmission route of SARS-CoV-2 by sharing utensils, food and beverages was recently suggested theoretically possible (Wendling et al., 2021). Furthermore, an oral transmission route for SARS-CoV-2 was suggested in an outbreak report from Thailand, where individual members of a group fell ill only after sharing a cup with a SARS-CoV-2-infected individual (Mungmungpantipantip and Wiwanitkit, 2020). Contradictory, other studies found no evidence of SARS-CoV-2 transmission via surfaces or drinking glasses in restaurant settings (Capon et al., 2021; Zhang et al., 2021). Syrian hamster model studies have shown, that oral administration of high amounts (10^5 infectious particles) of SARS-CoV-2 resulted in no clinical signs of disease, while intranasal inoculation resulted in ruffled fur, labored breathing, and loss of body weight. However, mild signs of infection were evident in the lungs of four out of six orally infected animals and both intranasal and orally infected hamsters shed equal amounts of virus post-infection (Lee et al., 2020). In human patients, the viral loads in saliva ranged from 10^4 to 10^8 virus copies/mL during the first week after onset of symptoms (Zhu et al., 2020). An additional study showed, that saliva of apparently healthy individuals can also contain a high viral load with a median of 1.1×10^6 genome copies/mL, and with a peak viral load of 6.1×10^{12} genome copies/mL in an individual who did not show symptoms at the time of testing (Yang et al., 2021). Thus, the contamination of dishes and glasses with a relatively high SARS-CoV-2 load through saliva might be possible.

From a public health perspective, it is important to ascertain that common cleaning procedures used in household and in food service settings are effective in eliminating SARS-CoV-2 to reduce the risk of virus transmission via this potential route. Previous studies have shown that SARS-CoV-2 is inactivated by heating to 56 °C for 15–30 min or to 65 °C for less than 15 min (Batejat et al., 2021). In line with this observation, virucidal effects of common household dishwasher machines on enveloped and non-enveloped viruses have been demonstrated recently (Lucassen et al., 2021). However, the high temperatures and specific cleaning reagents (e.g. 55 °C and bleach) used in dishwashing machines cannot be applied to manual washing procedures of dishes or glasses. It is therefore unclear, whether dish washing by hand using lower water temperatures and common household dish washing detergents will result in sufficient virus inactivation. Enveloped viruses are believed to be inactivated upon contact with detergent, as the lipid envelope will be disassembled. Accordingly, treatment with Triton X-100 resulted in successful SARS-CoV inactivation in the clinical sector (Rabenau et al., 2005a). However, a study found no titer reduction of infectious SARS-CoV-2 after incubation for 30 min in a 0.5% Tween 20 solution (Welch et al., 2020). Generally, data on stability of enveloped viruses in household detergent solutions are very limited and mainly focused on hand washing detergents with long contact times of up to 5 min (Lai et al., 2005; Sizun et al., 2000).

To address this data gap, the aim of this study was to assess the stability of HCoV in common household detergents and during a professional manual cleaning process for drinking glasses. HCoV-229E was used as a surrogate virus for SARS-CoV-2. The study included the investigation of virus stability on glass with and without daylight, inactivation by common household detergents at different temperatures and incubation times, and efficiency of a cleaning procedure using a portable manual glass washer. The generated data should contribute to an assessment of the efficiency of virus elimination through manual washing procedures of dishes and drinking glasses.

2. Methods and material

2.1. Cells and virus

HCoV-229E (ATCC® VR-740) was obtained from the American Type

Culture Collection (ATCC, Manassas, VA, USA) via LGC (LGC Standards GmbH, Wesel, Germany). The Huh-7 cell line was kindly provided by Christian Drosten (Charité, Institute of Virology, Berlin, Germany). All cell culture reagents and media were purchased from Pan Biotech (Aidenbach, Germany) unless otherwise indicated. Huh-7 cells were maintained at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM) with 1 x nonessential amino acids, 2 mM L-glutamine, and 0.1 µg/mL gentamicin (hereafter referred to as complete DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS). Cells were passaged twice a week in the ratio 1:8 after dislodging cells using 0.25% trypsin in EDTA. For virus propagation, 80% confluent Huh-7 cells were infected with HCoV-229E at multiplicity of infection of 0.1 for 1 h at 34.5 °C and 5% CO₂. The virus inoculate was subsequently removed and complete DMEM supplemented with 5% FCS was added. After 30 h, cells including culture supernatant were frozen at –80 °C, thawed at 37 °C, and cell debris was removed by centrifugation at 1000×g for 20 min and 4 °C. Bovine serum albumin (BSA) (Cell Signaling Technology, USA) solution prepared in tissue culture grade phosphate-buffered saline solution (PBS) and sterile filtered using a 0.22 µm PES membrane (Merck Millipore Ltd., USA) was added to the virus-containing supernatant to a final concentration of 3 g/L. This virus stock had a titer of 2.5×10^6 plaque forming units (PFU)/mL and was stored in 5 mL aliquots at –80 °C until use.

2.2. Plaque assay

Infectious virus particles were quantified by plaque assay in 6-well plates (VWR International, Darmstadt, Germany). Per well, 6.25×10^5 cells were seeded and propagated in 10% FCS complete DMEM. One day later, growth medium was removed and 500 µL of tenfold virus sample dilutions in DMEM were added to the wells. The plates were incubated at 34.5 °C and 5% CO₂ and manually rocked every 10–15 min. After 1 h, the virus inoculum was removed and the cell monolayer was covered with 3 mL overlay medium (previously held in a water bath at 40 °C). The overlay medium was composed of 1 x Eagle's Minimal Essential Medium, 1 x nonessential amino acids, 2 mM L-glutamine, 0.1 µg/mL gentamicin, 5% FCS, and 1% low gelling temperature agarose (Sigma-Aldrich, St. Louis, MO, USA). Plaque development was monitored under a light microscope on day 3 and cells were fixed overnight at 4 °C after adding 1 mL 10% formaldehyde solution (Carl Roth, Karlsruhe, Germany) on top of the agarose plug. Prior to staining, formaldehyde was aspirated and discarded and the agarose was removed. Thereafter, the fixed cells were covered with 500 µL crystal violet solution (Merck, Darmstadt, Germany) and incubated for 15 min while rocking the plate. Excess crystal violet was then removed (crystal violet solution was reused for up to 3 times), stained cells were washed with water and plates dried and stored in the dark until plaques were counted on a light table (Kaiser Fototechnik, Buchen, Germany). Throughout all plaque assays each virus sample was analyzed in duplicate. Virus titer in PFU/mL was calculated by multiplying the counted plaque numbers (mean value of the assayed duplicate) with the dilution factor and taking into account the volume of virus sample inoculum.

2.3. Virus infectivity after drying on glass

2.3.1. Sample preparation and drying on glass

Sterile glass slides (Menzel GmbH&Co KG, Braunschweig, Germany) were inoculated with 100 µL virus stock (corresponding to 2.5×10^5 PFU/glass slide), the aliquot left to dry within 1 h in a biosafety cabinet and transferred into a container for storage. At the indicated time-points, the remaining virus was removed from the slides using a sterile cotton swab (Paul Boettger GmbH&Co KG, Bodenmais, Germany) previously moistened with DMEM. The cotton tip of the swab was transferred into 700 µL DMEM in a 1.5 mL tube, mixed by vortexing for 15 s, and the tip removed with sterile forceps. Samples were stored at –80 °C until concentration of infectious virus was determined by plaque assay (2.2).

In a first experiment, virus titer was determined before and after drying on glass (within 1 h after virus sample was added to the glass slide) and at the following time points: 0h, 1h, 2h, 4h, 6h, 8h, 16h, and 24h in triplicate after incubation at room temperature in the dark (glass slides were stored in a light-sealed box). In a second experiment, infectious virus titer extracted from glass after drying was determined at the time points: 0h, 3d, 7d, 14 d, 21 d and 28 d at room temperature, in the dark (glass slides were stored in a light-sealed box) or in presence of indirect sunlight (glass slides were stored in a transparent glass box on a laboratory board in about 2 m distance from an east-facing window) in duplicates. Both experiments were performed in parallel and samples were collected simultaneously for both lighting conditions.

2.3.2. Virus detection limit after inoculation on glass

The virus stock was serially diluted in complete DMEM with 5% FBS and 3 g/L BSA, and 100 μ L aliquots were transferred to sterile glass slides. To exclude infectivity reduction by the drying process itself, the sample was not dried but taken 10 min after application with a cotton swab as described in 2.3.1. Subsequently, virus was removed and analyzed by plaque assay as described above (2.2). The amount of virus in the highest dilution detected by the plaque assay in at least one of three replicates was defined as the limit of detection, as previously described (Bartsch et al., 2016). The detection limit was determined in triplicate.

2.3.3. Virus recovery rate after inoculation on glass

To determine the recovery rate, the ratio of the virus titer extracted from sterile slides (without drying) and the virus titer of the stock solution was multiplied by 100%, as previously described (Blondin-Brosseau et al., 2021). The recovery rate was determined in triplicate.

2.4. Virus inactivation after exposure to dishwashing detergent

2.4.1. Virus infectivity after different durations and temperatures in dishwashing detergent

The HCoV-229E titer was determined by plaque assay after exposure to three different detergents at room temperature and at 43 °C for 15 s or 60 s in triplicate. The temperature of 43 °C had previously been used in a study to determine the effectiveness of manual dishwashing against bacterial survival (Lee et al., 2007), and is in line with a 2017 United States Food and Drug Administration (US FDA) Food code recommendation, that the temperature of wash solution in warewashing equipment shall be maintained at no less than 43 °C (FDA, 2017). Two different household dishwashing detergents were purchased at a local store. An additional beer glass washing detergent for manual glass washing was purchased from a specialized food service supplier. In addition, viral inactivation was determined in a solvent and detergent suspension (S/D) of Triton X-100 (Sigma-Aldrich) and tributylphosphate (Sigma-Aldrich) as used in clinical virology for the inactivation of viruses in plasma samples (Rabenau et al., 2005a). The surfactant composition declared on the dishwashing detergents as required by regulation on detergents (EC) No. 648/2004 annex VII varied among the purchased detergents as did the dose recommendation of the manufacturer (Table 1). Detergent solutions were prepared in sterile tap water. To test the virus inactivation in the different detergents, 50 μ L of virus stock (corresponding to $1,25 \times 10^5$ PFU) was added to 450 μ L of detergent solution, which had been equilibrated at room temperature or 43 °C in a water bath for at least 45 min prior to the start of the experiment. The final detergent concentration after addition of the 50 μ L aliquot corresponded to the manufacturer's recommended dose for the detergents or to the previously described virus inactivating concentration of the S/D (Rabenau et al., 2005a) as shown in Table 1. After 15 s or 60 s exposure time of the virus in detergent at room temperature or at 43 °C, the entire aliquot of 500 μ L was immediately added to a Detergent Removal Spin Column (Pierce™, ThermoScientific; Rockford, IL, USA), which was prepared with tissue culture PBS just prior to the detergent

Table 1

Recommended dose and composition of the detergents used for virus stability determination in detergent-containing liquids.

Detergent	Dose	Anionic surfactants	Non-ionic surfactants	Other components
1	3 mL/5L ^a ($\approx 0.06\%$)	15–30%	5–15%	–
2	5 mL/5L ^a ($\approx 0.1\%$)	5–15%	<5%	–
3	0.5–1 mL/ L ^a ($\approx 0.1\%$)	15–30%	–	2-Brom-2-nitro-1,3-propanediol Tributylphosphate
S/D ^c	1 % ^b	–	1%	

^a Dose as recommended by manufacturer.

^b Dose previously used for virus inactivation in clinical samples (Rabenau et al., 2005a).

^c S/D = solvent and detergent suspension of Triton X-100 and tributylphosphate.

experiment. Virus titer in the eluate was determined by plaque assay (2.2). For each condition, a control without adding detergent was included in triplicate, which was equally treated like the samples.

2.4.2. Virus detection limit

The virus stock was serially diluted in complete DMEM with 5% FBS and 3 g/L BSA and purified using Detergent Removal Spin Columns (Pierce™, ThermoScientific). Virus concentration in the eluate was determined by plaque assay. The amount of virus in the highest dilution detected by plaque assay in at least one of three replicates was defined as the detection limit, as previously described (Bartsch et al., 2016).

2.4.3. Virus recovery rate

To determine the recovery rate, the ratio of the virus titer of the eluate after purification using Detergent Removal Spin Columns (Pierce™, ThermoScientific) and the virus titer of the stock solution was multiplied by 100%, as previously described (Blondin-Brosseau et al., 2021).

2.5. Virus infectivity after manual washing of drinking glasses using a manual glass scrubbing device

An area of approximately 1.5 cm² was marked on the drinking rim of the outside of drinking glasses (ARCOROC, Arques, France) using a diamond pen. The marked glasses were placed on the side and an aliquot of 100 μ L virus stock (corresponding to 2.5×10^5 PFU/marked area) was added to the inside of each glass within the marked area and allowed to dry for 2 h. The glasses were then washed with a commercially available manual glass scrubbing and washing system, which is in accordance with the requirements of the German standard for manual glass scrubbing devices with physically separated pre-rinsing and post-rinsing canisters (DIN 6653–3:2011–01), which will hereafter be referred to as 'manual glass scrubbing device'. This device consisted of two physically separated pre-rinsing and post-rinsing canisters (Supplementary Material 1), with the pre-rinsing canister containing brushes and a detergent solution and the post-rinsing canister comprising horizontal freshwater jets. Six drinking glasses were washed using tap water at room temperature (23 °C) and six additional drinking glasses were washed using a water temperature of 18 °C (corresponding to the lowest tap water temperature reached in the laboratory). A detergent tablet provided by the manufacturer of the device was added to the pre-rinsing canister prior to the experiment. Additionally, the water pressure was adjusted, resulting in horizontal water jets in the post-rinse canister. For the washing process, the manufacturer's instructions were followed by cleaning the glass with four pumps on the brush in the pre-rinse canister without rotation and rinsing the glass in the second (post-rinse) canister by pressing the glass on the telescope rinse bar for 3 s. The glasses were thereafter air-dried and virus was recovered from the marked 1.5 cm²

area as described for the glass slides in 2.3.1. A positive control (no wash control) was included in triplicate, were the same amount of virus stock was dried on the glass rim and extracted without the washing process. The detection limit and recovery rate as determined in 2.3.2 and 2.3.3 also apply here, as the mechanism of virus extraction from drinking glasses and from glass slides as well as the plaque assay were the same. Photos of the experimental setup are shown in [Supplementary Material 1](#).

2.6. Statistical analysis and data presentation

All data was analyzed using Excel 2016 (Microsoft Corporation, Redmond, WA, USA) including determination of standard deviation. Single data points from virus infectivity study after drying on glass were plotted in addition to the mean values in a point-to-point graph. Single data points mean values obtained from the virus inactivation study after exposure to dishwashing detergent were plotted in dot plots.

3. Results

3.1. Virus stability after drying on glass

Virus stability on glass was assessed under conditions with access to daylight or in the dark after drying of HCoV-229E on glass slides. For the experimental procedure, an LOD of 75 PFU/glass slide and a recovery rate of $57 \pm 13\%$ were determined. Initial experiments indicated that the HCoV-229E mean titer did not decrease by more than $0.5 \log_{10}$ immediately after drying and within 24 h in the dark ([Supplementary Material 2](#)). Therefore, the change of the HCoV-229E titer was monitored for several days to weeks ([Fig. 1](#)). Under daylight conditions, the mean titer of infectious HCoV-229E decreased by $1.5 \log_{10}$ after three days, but then remained relatively stable until day 7. After 14 days, no remaining infectious virus was detected on the glass slides under these conditions. In the dark, the mean virus titer decreased by $0.5 \log_{10}$ after three days and remained stable until day 7 ([Fig. 1](#)). After 14 days, the mean virus titer was decreased by about $2 \log_{10}$ compared to day 0, and after 21 days, a HCoV-229E reduction of $>4 \log_{10}$ was observed. On day 28, no infectious HCoV-229E was detected on the glass slides under dark storage conditions. Considering the LOD of 75 ($1.87 \log_{10}$) PFU/glass slide determined for virus recovery from glass slides, exposure of dried HCoV-229E on glass slides for 14 days at daylight condition and for 21

days at dark condition resulted in mean titer reductions of $>2.8 \log_{10}$.

3.2. Virus inactivation by dishwashing detergents

The reduction of the HCoV-229E titer was analyzed after exposure to three different dishwashing detergents as well as S/D solution in aqueous suspensions at room temperature for 15 s ([Fig. 2A](#)) and 60 s ([Fig. 2B](#)) and at 43°C for 15 s ([Fig. 2C](#)) and 60 s ([Fig. 2D](#)). The LOD for the whole experimental process was 7.5 PFU/inoculum; the recovery rate was $82.67 \pm 21.00\%$. Using detergent 1, detergent 3 or S/D, the HCoV-229E mean titer decreased by $>4 \log_{10}$ at all tested conditions ([Table 2](#)). By use of detergent 2 at room temperature, a 1.31 and $2.88 \log_{10}$ reduction was observed after 15 s and 60 s, respectively. At 43°C the HCoV-229E mean titers were reduced by $3.68 \log_{10}$ and $>4 \log_{10}$ after incubation with detergent 2 for 15 s and 60 s, respectively. Considering the LOD of 7.5 PFU/mL, our results show that an HCoV-229E reduction of $>4 \log_{10}$ PFU/mL can be achieved with each of the three tested dishwashing detergents at 43°C for 60 s. The use of dishwashing detergents with higher surfactant content (detergents 1, 3 and S/D) lead to rapid HCoV-229E titer reductions with shorter contact time and lower temperature.

3.3. Virus elimination after washing with a commercial manual glass scrubbing device

The decrease of the HCoV-229E titer by the use of a commercial manual glass scrubbing device was investigated using virus-contaminated drinking glasses and water temperatures of 18°C or room temperature. After completion of the washing procedure, no remaining HCoV-229E was detected on the glasses at either temperature ([Table 3](#)).

4. Discussion

HCoVs including SARS-CoV-2 are mainly transmitted by aerosols and inhalation of virus-contaminated droplets ([Zhang et al., 2020](#)). However, other transmission routes, e.g. by contaminated dishes and drinking glasses, cannot be completely ruled out. In addition, from a public health perspective, it can be considered reasonable to demand the use of tableware and drinking glasses free of pathogenic viruses. In our study, we therefore investigated the stability of HCoV on glass and the

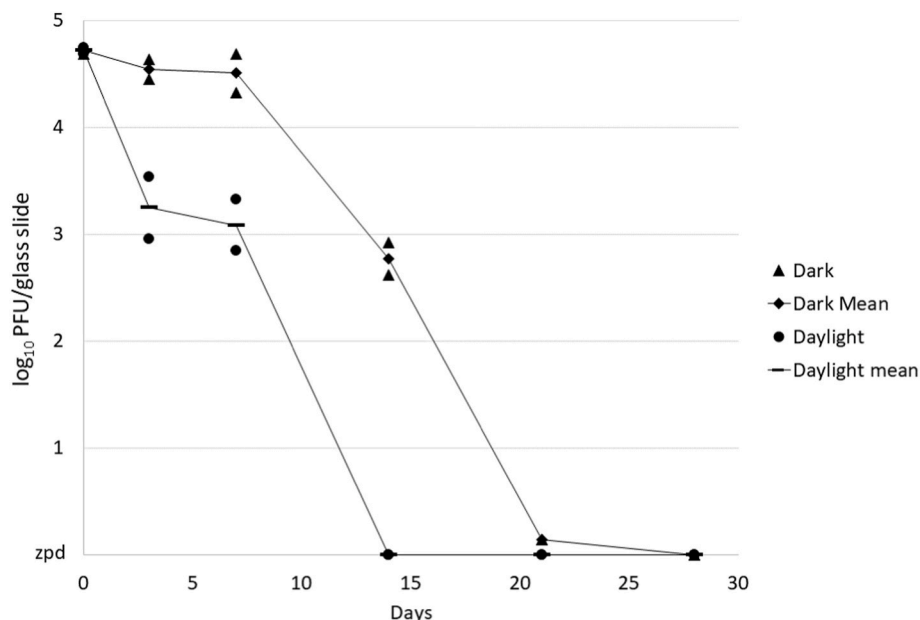


Fig. 1. Effect of storage at room temperature for up to 28 days on HCoV-229E titer after drying on a glass surface. HCoV-229E was extracted from glass slides after 0, 3, 7, 14, 21, and 28 days of storage in the daylight (round marker for single points and rectangle marker for mean values) and the dark (triangle marker for single points and diamond marker for mean values). Infectious HCoV-229E was determined by plaque assay and is shown in \log_{10} plaque forming units (PFU). Where data points are on the x-axis, zero plaques were detected (zpd).

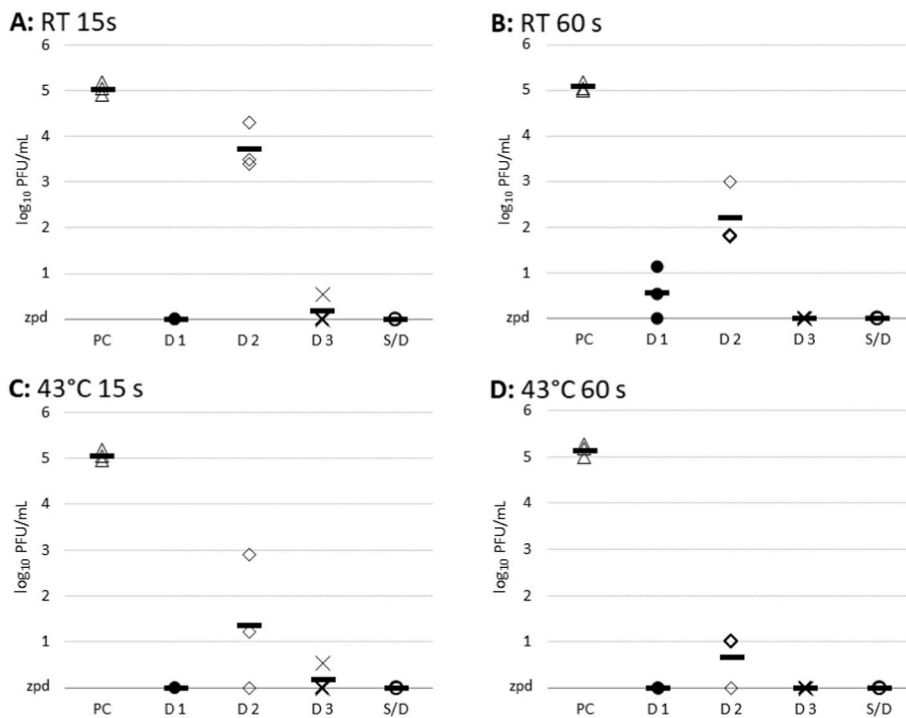


Fig. 2. Effect of three detergents (D1-D3), and solvent and detergent suspension (S/D) on HCoV-229E titer. Infectious HCoV-229E at room temperature (RT) after 15 s (A) and 60 s (B) incubation and at 43 °C after 15 s (C) and 60 s (D) incubation was determined by plaque assay and is shown in log₁₀ plaque forming units (PFU). For each condition a positive control (PC) was included. Where data points are on the x-axis, zero plaques were detected (zpd). Overlapping data points are indicated in bold and mean values (rectangle marker) are shown in addition to single data points.

Table 2

Reduction of HCoV-229E mean infectious titers after 15 s and 60 s exposure to different detergents at room temperature (RT) and 43 °C in log₁₀ PFU/mL.

Exposure condition	No detergent control (log ₁₀ [PFU/mL])	HCoV-229E reduction ^a after exposure to specified times and temperatures (log ₁₀ [PFU/mL])			
		Detergent 1	Detergent 2	Detergent 3	S/D
RT 15 s	5,04 ± 0.11	>4,17	1,31	4,86	>4,17
RT 60 s	5,07 ± 0.08	4,52	2,88	>4,20	>4,20
43 °C 15 s	5,06 ± 0.09	>4,19	3,68	4,87	>4,19
43 °C 60 s	5,15 ± 0.12	>4,28	4,48	>4,28	>4,28

^a The reduction was determined by subtracting the mean virus titer after detergent treatment from the mean virus titer of “no detergent control” and taking into account the LOD of 7.5 PFU (0.87 log₁₀).

Table 3

HCoV-229E mean titer and reduction after glass cleaning process using manual glass scrubbing device expressed as mean values of each six replicates at 18 °C and room temperature (RT) in log₁₀ PFU/mL.

Temperature	No wash control (log ₁₀ [PFU/mL])	Virus titer after wash (log ₁₀ [PFU/mL])	Reduction ^a (log ₁₀ [PFU/mL])
18 °C	4.74 ± 0.19	<1.87	>2.86
RT	4.74 ± 0.19	<1.87	>2.86

^a The reduction was determined by subtracting the mean virus titer after washing the glasses from the mean virus titer from the “no wash control” glasses after drying taking into account the LOD of 75 PFU (1.87 log₁₀).

elimination efficiency of common manual cleaning processes.

For this study HCoV-229E was used, for which reliable cell culture methods for infectivity titration are available and which can be handled under Biosafety Level (BSL)-2 laboratory conditions. Moreover, HCoV-229E has been previously used as surrogate virus for SARS-CoV and SARS-CoV-2 in several stability and inactivation studies (Blondin-Brosseau et al., 2021; Warnes et al., 2015), enabling direct comparisons with those data. The stabilities of different HCoVs on surfaces have been recently reviewed (Bueckert et al., 2020; Marzoli et al., 2021). By this,

differences between different coronaviruses as well as between different studies became evident. In most of these studies, SARS-CoV-2 was described as most resilient, being stable on glass surfaces for days to weeks (Pastorino et al., 2020; Riddell et al., 2020). However, in two studies, HCoV-229E has also been shown to stay infectious for several days after drying on glass surfaces (Bonny et al., 2018; Warnes et al., 2015). In both cases, the experiments were stopped after 5 or 7 days, leaving the time necessary for complete inactivation unknown. Besides possible differences between the distinct inactivation times, the relative contributions of environmental factors on stability seem to be similar for all HCoVs, e.g. low temperatures and/or low relative humidity usually increased their persistence (Bueckert et al., 2020). Therefore, although slight differences in inactivation of HCoV-229E and SARS-CoV-2 have to be expected, the general conclusions drawn from our study regarding inactivation by detergents and during manual washing procedures should apply to both viruses.

For the method applied in our stability study on glass, an approximate LOD of 75 PFU (1.87 log₁₀) was determined, which is comparable to LODs of about of 50–125 PFU and about 1.8 log₁₀ TCID₅₀/mL described in similar previous studies (Blondin-Brosseau et al., 2021; Rabenau et al., 2005b). Using this method, we detected infectious virus for up to 21 days after drying on glass, albeit with a very low titer. Previous studies investigated HCoV reduction times after drying on glass found a 3.5 log₁₀ reduction for SARS-CoV-2 in seven days or 2 log₁₀ reduction for HCoV-229E in five days (Liu et al., 2021; Warnes et al., 2015). However, in these studies the light conditions (daylight or darkness) during the experiments were not specified. In our study, exposure to daylight shortened the virus stability on glass, with 7 day as the longest time-interval for detection of infectious virus. After these 7 days a virus reduction of about 1.5 log₁₀ compared to the original inoculum was observed. An additional study investigating HCoV-229E in classroom conditions using fluorescent lights for 14 h/d found a similar reduction of 2.5 log₁₀ in the same time frame, where the virus inactivation was stopped after 7 days and the last virus concentration determined was about 2 log₁₀ (Bonny et al., 2018). The results of other studies confirm the contribution of UV light and artificial sunlight to HCoV titer reduction (Heilingloh et al., 2020; Ratnesar-Shumate et al., 2020). One study, assessing the HCoV stability in the dark, recovered

infectious SARS-CoV-2 after drying on glass until 28 days of storage at 20 °C, with a 4 log₁₀ reduction in virus titer (Riddell et al., 2020), which is in line with our observations. It can be concluded from the data of the stability experiments, that HCoVs are relatively stable after drying on glass. This is also reflected in previous studies assessing the stability of coronaviruses on glass, where viable virus is generally recovered after days to weeks (Bonny et al., 2018; Chan et al., 2020; Riddell et al., 2020; Warnes et al., 2015) depending on temperature among other factors. Therefore, residual infectious virus on contaminated drinking glasses and dishes must be expected to remain for several days, if appropriate cleaning regimes are not applied.

The efficiency of manual dishwashing procedures is considered to be mainly dependent on the detergent used, the temperature of the wash water and the contact time of virus and detergent. We investigated the inactivation of HCoV-229E in aqueous suspension with three household detergents and the control S/D at two temperatures for 15 s and 60 s. The detergent removal method using spin columns yielded a HCoV-229E recovery rate of 82%, which is comparable to a similar method reported for SARS-CoV-2 (Welch et al., 2020), and enabled the determination of virus inactivation within a 4 log₁₀ range. German guidelines for disinfectants specify, that in suspension a sufficient virus inactivation can be assumed, if the virus titer is reduced by at least 4 log₁₀ (EN 14476:2013+A2:2019; Rabenau et al., 2015). In our study, we could show that two of the household detergents had those inactivating activities (>4 log₁₀) on the HCoV-229E, even at room temperature and with a short contact time of 15 s. For sufficient virus inactivation using detergent 2, however, 43 °C and a contact time of 60 s was necessary. The main difference between the detergents is the percentage of anionic detergents present in the formulations, which was 15–30% for the efficiently inactivating household detergents and 5–15% for detergent 2. Further studies should examine if higher concentration of detergent 2 or a longer contact time will result in a more efficient inactivation of HCoV-229E, or whether other variations in the ingredients of the tested detergents are the cause for the difference. The results of our study obtained for HCoV-229E with the control S/D are similar to those described for SARS-CoV, which resulted in a >4.5 log₁₀ reduction after 1 min (Rabenau et al., 2005a). Another recent study confirmed the inactivation of SARS-CoV-2 using a 0.5% Triton X-100 solution achieving a 5.9 log₁₀ reduction after exposure of less than 2 min (Welch et al., 2020). Only a few studies have assessed the efficiency of household detergents for HCoV inactivation. Lai et al. (2005) reported a reduction of >3 log₁₀ for SARS-CoV after exposure of to a 1:100 dilution of a household detergent for 5 min. From our study and the published data, it can generally be concluded that household detergents inactivate HCoVs in solution. However, differences in efficiency exist between different products, and inactivation efficiency is affected by both temperature and contact time.

In addition to the detergent, mechanical removal of contaminants from the surface and dilution in the washing water represent important factors during a complete washing procedure of dishes and drinking glasses. In order to test the efficiency of a whole washing procedure for drinking glasses under more realistic conditions, a commercially available manual glass scrubbing device was used in our study. This device was in accordance with DIN 6653-3 (2011) performance requirements, which guarantees high cleaning efficiencies due to its construction. In the device, virus is mechanically eluted from the glass by scrubbing in the pre-rinse canister, where eluted virus is exposed to detergent. The glass is subsequently transferred to the post-rinse canister, where the glass is rinsed with fresh tap water to remove detergent and loosened dirt. We did not detect any remaining virus on the initially HCoV-229E-inoculated glasses after completion of the washing process. However, the variation of the applied parameters such as shorter washing times and lower flow-through of fresh water, but also the presence of higher concentrations of protein or fat, may reduce the elimination efficiency of viruses.

Our study has some limitations. First, as discussed above, it was

conducted with HCoV-229E as surrogate virus for SARS-CoV-2, and confirmation with SARS-CoV-2 would be desirable in future investigations. Second, the virus was only tested in culture medium with BSA, but not in other matrices. Although the use of this standardized solution enables comparison with data from similar studies, it does not fully reflect conditions in real-life, and negative effects on virus inactivation in complex matrices have been described (Bertrand et al., 2012). Therefore, studies investigating virus reduction in complex matrices such as saliva or specific beverages would be useful, although a wide variety of other matrices may be considered. Third, only a small spectrum of detergents and only one glass scrubbing device was tested here, which should be extended in further studies.

In summary, the results of our study indicate that HCoV-229E can stay infectious for days to weeks after drying on glass, where daylight exposure contributes to a more rapid inactivation. Sufficient cleaning of dishes and drinking glasses is therefore crucial. Common household detergent solutions can inactivate the virus, some of them with high efficiency even at room temperature and with low contact times. However, as one tested dishwashing detergent with lower overall surfactant content showed lower efficiency, the use of higher temperatures and longer contact times should generally be suggested for manual dish washing procedures. Finally, it was shown that a commercial glass scrubbing device can be used for efficient elimination of HCoV-229E from drinking glasses. Future studies should investigate the influence of variations in washing procedures used and the influence of protein/fat contamination on virus elimination efficiency to provide further data for risk assessment under more variable real-life conditions, which can be used to propose commercial and household cleaning processes.

Declaration of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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