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Dried SARS-CoV-2 virus maintains infectivity to Vero E6 cells for up to 48 h

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ABSTRACT

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is a great concern on both public and veterinary health. Multiple studies showed that the SARS-CoV-2 can persist for few days in wet condition, but it has not been clear whether the virus can maintain the infectivity in dry condition. Thus, we measured the infectious titer of dried SARS-CoV-2 (10^4 pfu/25 μL droplet) at 0, 0.5, 1, 2, 3, 24, and 48 h. Strikingly, the dried SARS-CoV-2 virus did not lose the infectivity to Vero E6 cells for up to 48 h. Our findings warrants that the drying cannot replace the surface disinfection to prevent transmission via common vehicle or nosocomial infection. Future studies can apply our experimental setting to test the efficacy of diverse disinfecting procedures.

The world is experiencing an intensive pandemic (COVID-19) caused by a novel zoonotic severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The SARS-CoV-2 appear to be originally derived from bat, and pangolins might serve as a intermediate host to deliver the virus into human (Yoo and Yoo, 2020; Zhou et al., 2020). Also, multiple studies confirmed that the SARS-CoV-2 can cause the reverse-zoonotic infection of pet animals, such as cats, dogs, hamsters and minks (Molenaar et al., 2020; Stout et al., 2020; Yoo and Yoo, 2020). Thus, control of SARS-CoV2 is a great issue to both public and veterinary health.

The world-wide lockdown and quarantine procedures could not stop the spread of SARS-CoV-2. Multiple factors may contribute to the rapid spreading of this virus, such as viral shedding from asymptomatic patients, lack of pre-existing immunity, and high stability of the virus.

Several studies have reported that SARS-CoV-2 does not lose its infectivity in diverse environments for days, especially on glass surfaces (World Heath Organization, 2020). According to an early report from the World Health Organization, SARS-CoV-2 collected from sterilized stool was infectious for up to four days on glass slides. In a different setting, the half-life of free SARS-CoV-2 was 1.2 h on a glass surface, which is similar to its half-life on a mask (1.0–1.4 h) or a plastic surface (1.6 h) but much longer than its half-life on stainless steel (0.3 h) or a banknote (Chin et al., 2020). Pastorino et al. verified that the presence of BSA could substantial prolong the infectivity of SARS-CoV-2 on glass, plastic, and aluminum surface (Pastorino et al., 2020). As the presence of BSA mimics the environment of body fluids, such as cough droplets, sputum, and airway mucus secretions, the study result warrants that the SARS-CoV-2 virus contained in body fluid could persist longer than 100 h (Pastorino et al., 2020).

These early reports emphasize the need for surface decontamination to prevent the spreading of SARS-CoV-2. However, those studies were conducted on viruses in liquid, and might not truly represent dry environment where nosocomial or contact transmission occurs.

Table 1
Stability of SARS-CoV-2 air-dried at different time points.

Time	Virus titer[†]					
	Log ($TCID_{50}$/mL)		Log (PFU/mL)[‡]		Previous report (Chin et al) Log ($TCID_{50}$/mL)	
	Mean	±SD	Mean	±SD	Mean	±SD
Stock	6.1	0	5.69[‡]	–	~7·8	–
0 hr	5.23	0.18	4.00	0.17	5.83	0.04
.5 hr	5.10[‡]	–	N.D.	N.D.	5.81	0.27
1 hr	5.23	0.18	N.D.	N.D.	N.D.	N.D.
2 hrs	4.48	0.18	N.D.	N.D.	N.D.	N.D.
3 hrs	4.23	0.18	4.88	0.09	5.14	0.05
24 hrs	3.10	0.35	3.22	0.12	3.48	0.37
48 hrs	1.23	0.18	N.D.	N.D.	2.44	0.19

[†]All the virus titers were titrated using Vero-E6 cells. All $TCID_{50}$/mL was calculated in this study is based on two independent quadruplicates unless indicated otherwise[‡]. The PFU/mL was based on one experiment with triplicates.
[‡]Value obtained from one trial N.D.: not done.

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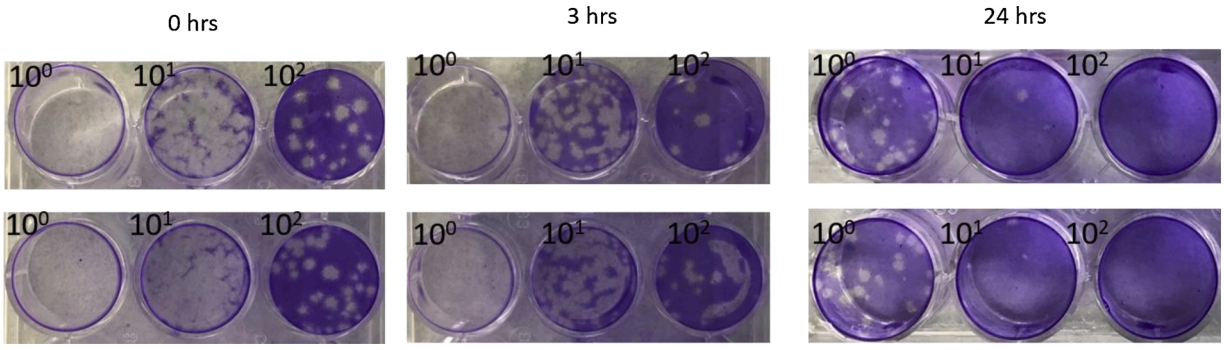

Fig. 1. Reduction of plaque forming unit of dried SARS-CoV2 droplet (25 μl before dry) by time. Representative plaque plates. Dried SARS-CoV-2 virus (~10^4pfu) was resuspendded with 100 μl of 1 % FBS containing DMEM at 0, 3, and 24 h. The virus suspension was 10-fold diluted and used for the plaque assay performed on monolayer of Vero E6 cells seeded on 12 well cell culture plate (~5 × 10^4/well).

We investigated the stability of SARS-CoV-2 on a dry surface, particularly on glass. As a non-porous material, glass is commonly used as bowl for feed or water, and caging which can act as a vehicle for the transmission of SARS-CoV2. The SARS-CoV-2 infectivity was maintained in glass for longer than that in other types of surfaces, such as aluminum. Further, a glass surface was more appropriate for the development of a standardized methodology by simply using glass slides that are commonly used for histology analyses.

Briefly, prior to the experiment, the backside of a glass slide was divided into four equal sections (about 35 mm by 7.5 mm) using ImmEdge Hydrophobic Barrier PAP Pen (Vector Laboratories, USA). The SARS-CoV-2 stock solution from passage 2 was diluted to 10^4 pfu/25 μL in 1 % FBS-containing DMEM, and a 25-μL droplet of the diluted virus suspension was placed on one section of the prepared and previously sterilized glass slide (approximately 10 mm × 35 mm; Fig. S1). To facilitate drying, the droplet was spread within the pre-defined space. The thin layer of diluted virus suspension was left to completely dry inside the biosafety cabinet for about 40 min under the conventional florescence lamp. The glass slide was then moved onto a table in the biosafety level-3 laboratory. The room temperature and humidity were maintained at around 20 °C and 25 %, respectively, and the air change was approximately 30 times/h. At 0, 0.5, 1.2, 3, 24, and 48 h, the dried virus layer was resuspended in 100 μL 1 % FBS-containing DMEM. To calculate the median tissue culture infectious dose ($TCID_{50}$), the resuspended virus was diluted 10-fold and inoculated onto 5 × 10^4 Vero E6 cells seeded in 96-well cell culture plates. All time points were assessed in quadruplicate and all the procedures were repeated twice. In addition, we repeated the same procedure at 0, 3, and 24 h to measure the plaque-forming unit.

We found that SARS-CoV-2 maintained its infectivity for up to 2 days, despite the dry condition (Table 1). The virus titer right after drying (at 0 h) was only one log lower to that in the original stock solution for (Table 1). The titer was maintained as around 10^5 $TCID_{50}$/mL for 1 h, and only one log reduction was observed after 3 h. By plaque forming unit, no reduction was observed for up to 3 h. At 24 h, the virus titer was around 10^3 $TCID_{50}$/mL or pfu/mL (Fig. 1). Even after 48 h, the virus titer was still detectable. The half-life of dried SARS-CoV-2 was calculated as 1.331 h using PRISM GraphPad Software (San Diego, CA, USA) (Fig. S2). Strikingly, the virus titer observed from this study was very close to the previous report by Chin et al., except that the initial viral stock was about one log lower in this study (Table 1) (Chin et al., 2020).

It was common understanding that enveloped viruses, such as SARS-CoV-2, are more vulnerable to dry conditions than non-enveloped viruses. Firquet et al. compared the persistence of enveloped viruses (influenza and herpes simplex viruses type 1 [HSV-1]) with that of non-enveloped viruses (minute virus of mice and coxackievirus B4) in an experimental setting similar to ours (Firquet et al., 2015). After drying on a petri-dish, the influenza virus and HSV-1 could maintain their infectivity for up to 2 and 3 days, respectively. This was lower than that observed for the non-enveloped viruses, which maintained their infectivity for 5 weeks or longer.

As a common belief, the enveloped viruses will decay just by drying process. Our findings emphasizes the importance of frequent surface decontamination. For our knowledge, the stability of dried SARS-CoV-2 has not previously investigated. The most similar study was conducted in 2010, with SARS-CoV-1 virus (Chan et al., 2011). The SARS-CoV dried on plastic surface (representing a nonporous surface) showed only one log reduction during incubation at 22~25 °C at relative humidity of 40~50 % for five days (Chan et al., 2011). Interestingly, the viral infectivity dropped faster at high temperature high temperature (38 °C) and high relative humidity (>95 %). The viral stability was not significantly influenced by the high relative humidity at a low temperature, or the high temperature at a relatively lower humidity.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2020.108907.

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