

1 **Running title:** Stability of SARS-CoV-2 on surfaces

2 **Keywords:** SARS-CoV-2, environmental stability, fomite, virus decay

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4 **Title:** Environmental stability of SARS-CoV-2 on different types of surfaces under indoor  
5 and seasonal climate conditions

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10

11 **Abstract – 50 words**

12 We report the stability of SARS-CoV-2 on various surfaces under indoor, summer and  
13 spring/fall conditions. The virus was more stable under the spring/fall condition with virus  
14 half-lives ranging from 17.11 to 31.82 hours, whereas under indoor and summer conditions  
15 the virus half-lives were 3.5–11.33 and 2.54–5.58 hours, respectively.

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17 **Text – 1167 words**

18 **The Study**

19 Severe acute respiratory coronavirus 2 (SARS-CoV-2) which first emerged in a wet market in  
20 Wuhan, China, is responsible for the current pandemic. Although transmission of SARS-  
21 CoV-2 mainly occurs through infectious droplets or close contact with an infected person, the  
22 virus droplet can survive and remain infectious on inanimate surfaces, which can contribute  
23 to the spread of the virus (1). Previous studies showed that virus remained infectious from  
24 hours to days on various type of surfaces under various temperature-controlled environmental  
25 conditions (2-4). However, virus stability on surfaces under different climate conditions  
26 which could be used to predict seasonality of SARS-CoV-2, is poorly understood. In this  
27 manuscript, we evaluated the stability of SARS-CoV-2 on different types of surfaces under  
28 indoor, summer and spring/fall conditions to estimate the biological half-life of the virus.

29 We tested SARS-CoV-2 stability on 12 material surfaces including nitrile glove, Tyvek, N95  
30 mask, cloth, Styrofoam, cardboard, concrete, rubber, glass, polypropylene, stainless steel and  
31 galvanized steel (see Technical Appendix). Each material surface was placed in a 6-well or  
32 12-well plate and 50  $\mu$ l of virus inoculum consisting of  $5 \times 10^4$  TCID<sub>50</sub> SARS-CoV-2 (strain  
33 USA-WA1/2020) in DMEM with 5% FBS was added onto each material. The positive  
34 control had the same amount of virus in medium in a sealed 2mL tube. The virus was air-  
35 dried inside a biosafety cabinet (approximately 4.5 hours). The plate with the virus-  
36 contaminated material was incubated under three different conditions: 21°C/60% relative  
37 humidity (RH), 25°C/70% RH and 13°C/66% RH, environmental conditions simulating  
38 indoor setting, summer, and spring/fall conditions for the Midwestern U.S., respectively  
39 (Technical Appendix Table 1). At each time point indicated, infectious virus was recovered in  
40 2 mL media through vigorous vortexing for 10 seconds. Cardboard was soaked with media

41 for 5 minutes and vortexed for 10 seconds. The recovered virus was titrated on Vero E6 cells  
42 and virus titer was calculated by the Reed-Muench method. The assay was performed in  
43 triplicate. A best-fitting line was estimated using a linear regression model in order to  
44 calculate the virus half-life on each surface as a  $-\log_{10}(2)/\text{slope}$  and tested for statistical  
45 significance using default analysis which is compatible to analysis of covariance in GraphPad  
46 Prism 5.

47 SARS-CoV-2 was relatively stable in medium throughout the study phase, showing a 1.17-  
48 log reduction of virus titer at 96 hours post-contamination (hpc) at 25°C/70% RH (Figure 1).  
49 We found a 1-log reduction of virus after 4.5 hours at room temperature (21°C/60% RH) on  
50 all materials ( $10^{3.3}$  to  $10^{4.2}$  TCID<sub>50</sub>), except for cloth ( $10^{2.4}$  to  $10^{2.7}$  TCID<sub>50</sub>), which served as  
51 the starting titers for the linear regression model. At 21°C/60% RH, infectious virus was  
52 recovered from cloth up to 24 hpc, from concrete, polypropylene, stainless steel and  
53 galvanized steel up to 72 hpc, and from nitrile gloves, Tyvek, N95 mask, Styrofoam,  
54 cardboard, rubber and glass up to 96 hpc. In contrast, viable virus disappeared quickly under  
55 summer conditions (25°C/70% RH) and was undetectable on cloth, cardboard, concrete and  
56 stainless steel at 48 hpc, and on nitrile gloves, Tyvek, N95 mask, Styrofoam, rubber, glass,  
57 polypropylene, galvanized steel at 72 hpc. However, we observed longer survival times at  
58 spring/fall conditions (13°C/66% RH). Virus titers on surfaces ranged from  $10^{1.1}$  to  $10^{2.3}$   
59 TCID<sub>50</sub> at 168 hpc, except for cloth with virus only detectable up to 72 hpc. Half-lives of  
60 SARS-CoV-2 on surfaces ranged from 3.5 to 12.86 hours at 21°C/60% RH, 2.54 to 5.58  
61 hours at 25°C/70% RH, and 17.11 to 31.82 hours at 13°C/66% RH (Table 1). The virus  
62 survived significantly longer on all surfaces at spring/fall conditions (13°C/66% RH) when  
63 compared to summer and indoor conditions. Similarly, we found a significant difference in  
64 virus survival on surfaces between indoor and summer conditions except for cloth.

65 Potential modes of transmission of SARS-CoV-2 include direct contact with an infected  
66 person via droplets, inhalation of aerosol or infectious body fluids, and exposure to  
67 contaminated surfaces (fomite). To date, there is no scientific report which demonstrates  
68 SARS-CoV-2 infection via contaminated surfaces. However, the role of fomites in  
69 transmission of SARS-CoV-2 is debated because the virus has been detected on  
70 environmental surfaces as well as personal protective equipment in hospitals and households  
71 (5, 6). In addition, indirect transmission of SARS-CoV-2 has been supported by a cluster of  
72 SARS-CoV-2 infection cases in a shopping mall, in which contact tracing failed to find any  
73 evidence for direct contact to an infected person, only to sharing of facilities (7). In this  
74 respect, our study highlights the possible role of contaminated surfaces in SARS-CoV-2  
75 transmissions because SARS-CoV-2 remained viable and infectious on surfaces for 1 to 4  
76 days at indoor conditions (21°C/60% RH), 1 to 3 days during summer conditions (25°C/70%  
77 RH) and over 7 days during spring/fall conditions (13°C/66% RH).

78 Van Doremalen et al. (3) described that the SARS-CoV-2 half-life which ranges from 3.46 to  
79 6.81 hours on cardboard, plastic and stainless steel at 22°C/40% RH. Chin et al (2) reported a  
80 half-life of 4.8 to 23.9 hours on glass, banknotes, inner and outer mask layers, polypropylene  
81 and stainless steel at 22°C/65% RH. We found the half-life on most surfaces at 21°C/60% RH  
82 is 6.93–12.86, but the virus is quickly inactivated on cloth with a 3.5 hours half-life. The  
83 difference might be explained by the composition of the virus inoculum (e.g., FBS  
84 concentration), the volume of inoculum, different preparation of material and the different  
85 environmental conditions. However, our results, along with other two studies, showed that  
86 SARS-CoV-2 is able to survive on some surfaces for several days under indoor conditions,  
87 which might play a potential role in virus transmission. The longest half-life of the virus was  
88 found in spring/fall conditions (13°C/66% RH), followed by indoor conditions (21°C/60%

89 RH) and summer conditions (25°C/70% RH); this suggests that virus stability on surfaces is  
90 highly dependent on temperature and RH. Prolonged virus survival on surfaces in spring/fall  
91 and winter might support SARS-CoV-2 transmission through contaminated fomites and  
92 potentially contribute to new outbreaks and/or seasonal occurrence in the post-pandemic era,  
93 a scenario described for influenza virus and other human coronaviruses (8).

94 Our study showed a remarkable persistence of infectious SARS-CoV-2 on various types of  
95 surfaces, especially under spring/fall climate conditions. However, virus stability was highly  
96 dependent on the substrate as well as temperature and humidity. Previous studies showed  
97 reduced virus stability in human nasal mucus and sputum when compared to culture medium  
98 (9) even at 4°C/40% RH, whereas addition of bovine serum albumin into the virus inoculum  
99 increased SARS-CoV-2 survival times (10). In addition, exposure to simulated sunlight  
100 accelerated the inactivation of the virus on stainless steel (11), indicating that additional  
101 factors play a role in SARS-CoV-2 survival on surfaces in field settings.

102 In conclusion, our study determines the half-life of SARS-CoV-2 on diverse surfaces under  
103 different climatic conditions, which correlates to the potential risk of contaminated surfaces  
104 to spread the virus. It clearly demonstrates, that the virus survives longer under spring/fall not  
105 summer conditions. Therefore, practice of good personal hygiene and regular disinfection of  
106 potentially contaminated surfaces remains a critical tool to minimize the risk of infection  
107 through contaminated surfaces.

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117 **Biographical Sketch**

118 Mr. Kwon is a Doctor of Veterinary Medicine and presently a PhD student at Kansas State  
119 University. His research interest are transboundary animal disease and emerging zoonotic  
120 diseases.

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158 **Figure captions**

159 **Figure 1.** Stability of severe acute respiratory coronavirus 2 (SARS-CoV-2) on different  
160 types of surfaces. Each figure represents the virus decay on each surface. Total 50  $\mu$ l of virus  
161 inoculum ( $5 \times 10^4$  TCID<sub>50</sub>, black dot) was added onto each material and dried for 4.5 hours  
162 inside a biosafety cabinet. The virus survival was evaluated under three different conditions:  
163 at 21°C/60% RH (grey), 25°C/70% RH (red) and 13°C/66% RH (green). The infectious virus  
164 was recovered at 4.5 (after drying period), 24, 48, 72, and 96 hours post-contamination (hpc)  
165 at 21°C/60% RH and 25°C/70% RH and 4.5, 24, 72, 120, and 168 hpc at 13°C/66% RH.  
166 Virus titer at each time point was expressed as mean log<sub>10</sub> transformed titer with standard  
167 deviation. Linear regression models were estimated; the solid line and its shade area represent  
168 an estimated best fit model and 95% confidence intervals, respectively. Limit of detection  
169 (LOD) in each titration assay was  $10^{0.968}$  TCID<sub>50</sub> and a negative result is represented as a half  
170 value of LOD,  $10^{0.667}$  TCID<sub>50</sub>. The dash line shows LOD in triplicate,  $10^{0.767}$  TCID<sub>50</sub>, when  
171 there was LOD in one replicate, but negative in two other replicates. Statistical significance  
172 between two slopes of linear regression models is represented as \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ),  
173 \*\*\* ( $p < 0.001$ ).

174 **Table 1. Half-lives of severe acute respiratory coronavirus 2 (SARS-CoV-2) on different types of surfaces. The virus decay rates were**  
 175 **evaluated under three different conditions, 21°C/60% RH, 25°C/70% RH and 13°C/66% RH, which simulate indoor, summer and**  
 176 **spring/fall conditions, respectively.**

Surface materials	21°C, 60% relative humidity (indoor condition)			25°C, 70% relative humidity (Summer condition)			13°C, 66% relative humidity (Spring/fall condition)		
	Half-life (hours)	95% confidence interval (hours)	$r^2$	Half-life (hours)	95% confidence interval (hours)	$r^2$	Half-life (hours)	95% confidence interval (hours)	$r^2$
Nitrile gloves – outer surface	11.56	8.27, 19.21	0.69	4.42	3.5, 6.03	0.92	22.94	18.73, 29.63	0.88
Tyvek	9.36	7.76, 11.79	0.89	4.57	3.84, 5.63	0.96	31.82	24.65, 44.82	0.81
N95 mask	9.01	7.57, 11.12	0.91	4.4	3.64, 5.57	0.95	27.77	22.5, 36.27	0.87
Cloth	3.5	2.77, 4.75	0.97	2.99	2.45, 3.84	0.98	19.94	13.94, 34.95	0.81
Styrofoam	9.62	8.04, 11.98	0.9	4.75	3.73, 6.53	0.92	24.67	20.6, 30.73	0.9
Cardboard	12.86	10.52, 16.54	0.88	5.03	3.5, 8.95	0.91	26.93	23.55, 31.42	0.95
Concrete	7.96	5.25, 16.44	0.65	2.54	1.55, 6.98	0.83	17.11	14.38, 21.14	0.91
Rubber	11.33	8.95, 15.45	0.83	5.03	3.63, 8.18	0.84	28.27	22.4, 38.32	0.84
Glass	9.6	8.05, 11.89	0.91	5.58	4.72, 6.82	0.96	27.34	21.72, 36.87	0.84
Polypropylene	9.02	7.22, 12.03	0.89	4.51	3.74, 5.68	0.95	28.75	21.52, 43.36	0.76

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Stainless	7.75	6.39, 9.86	0.92	3.41	2.36, 6.16	0.91	23.46	20.16, 28.08	0.93
Galvanized	6.93	5.88, 8.43	0.94	4.19	3.68, 4.85	0.98	24.22	21.3, 28.08	0.95
Positive control	35.54	23.19, 75.88	0.56	29.48	20.85, 50.39	0.68	100.68	52.35, 1346.89	0.3

179 **Environmental stability of SARS-CoV-2 on different types of surfaces under indoor and seasonal climate conditions**

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181 **Technical appendix**

182

183 **Preparation of surface materials**

184 Materials used in this study were nitrile glove (Kimberly-Clark Professional™ Kimtech™ G3 Sterile Sterling™ Nitrile Gloves), Tyvek  
185 (DuPont™ Tyvek IsoClean Sleeves. Clean Processed & Sterile, White), N95 mask (3M N95 mask 1870), cloth (65% polyester and 35%  
186 cotton from local source), styrofoam (50mL centrifuge tube-foam rack, CELLTREAT Scientific Products), cardboard (inner packing, TPP T75  
187 flask), concrete (Fast-setting concrete mix, The Home Depot), rubber (The Home Depot), glass (Electron Microscopy Sciences),  
188 polypropylene (biohazard autoclave bag, ThermoFisher), stainless steel (Metal Remnant Inc.), and galvanized steel (The Home Depot).  
189 Materials were cut into small pieces, washed, dried and autoclaved (depending on material). To make concrete, the coarse aggregate was  
190 removed by a strainer, and the fine aggregate was mixed with water according to the manufacturer's instruction. Mixture was poured into a  
191 silicone mold and air-dried in biosafety cabinet overnight.

192

193 **U.S. Midwest climate conditions**

194 Maximum and minimum temperature and relative humidity (RH) data at Manhattan, Kansas, was acquired from National Service Forecast  
195 Office on 5/11/2020 (<https://w2.weather.gov/climate/index.php?wfo=top>). Average temperature and RH was calculated for each season.  
196 Climate conditions for spring and fall were combined since their average temperature and RH were similar. Spring/fall and summer conditions  
197 were 13°C/66% RH and 25°C/70% RH, respectively.

198 **Technical Appendix Table 1.** Maximum and minimum temperature and relative humidity data for Manhattan, Kansas

Season	Spring		Summer		Fall		Winter		Spring			
	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Month and year	2019	2019	2019	2019	2019	2019	2019	2019	2020	2020	2020	2020
Maximum temperature (°F)	73.6	86.9	91.8	86.7	88.1	63.7	54.5	48.6	42.8	47.5	60.1	67.9
Minimum temperature (°F)	52.9	61.9	68.3	68	65.9	39	27.2	23	22.3	21.8	37	39.8
Relative humidity (%)	73	67	66	76	69	66	60	68	73	61	67	59

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