

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.

16

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 µl of virus inoculum consisting of 5×10^4 TCID₅₀ 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 $10^{4.2}$ TCID₅₀), except for cloth ($10^{2.4}$ to $10^{2.7}$ TCID₅₀), 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 TCID₅₀ 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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Biographical Sketch

Mr. Kwon is a Doctor of Veterinary Medicine and presently a PhD student at Kansas State University. His research interest are transboundary animal disease and emerging zoonotic 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 µl of virus
161 inoculum (5×10^4 TCID₅₀, 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 log₁₀ 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 $10^{0.968}$ TCID₅₀ and a negative result is represented as a half
170 value of LOD, $10^{0.667}$ TCID₅₀. The dash line shows LOD in triplicate, $10^{0.767}$ TCID₅₀, 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

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

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

Technical appendix

Preparation of surface materials

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

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		
Month and year	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
	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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