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Persistence of SARS-CoV-2 on surfaces and relevance to the food industry

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Determining the prevalence and persistence of viruses outside the human host aids our ability to characterize exposure risk across multiple transmission pathways. Since 2020, the Coronavirus Disease 2019 pandemic has resulted in a surge of research regarding severe acute respiratory syndrome-coronavirus-type 2 (SARS-CoV-2) and its potential to spread via direct and indirect contact transmission routes. Here, the authors discuss the current state of the science concerning SARS-CoV-2 transmission via contaminated surfaces and its persistence on environmental surfaces. This review aims to provide the reader with an overview of the currently published SARS-CoV-2 persistence studies, factors impacting persistence, guidelines for performing persistence studies, limitation of current data, and future directions for assessing SARS-CoV-2 persistence on fomites.

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Introduction

When severe acute respiratory syndrome (SARS) coronavirus (CoV) type 2 (SARS-CoV-2) was identified as the causal agent of COVID-19 (Coronavirus Disease 2019) in early January 2020 [1], knowledge regarding the transmission of this novel CoV and its potential impact on the food industry was limited. In the early months of the COVID-19 pandemic, the primary focus was placed on advancing research related to the clinical aspects (i.e. diagnosis and treatment) of SARS-CoV-2. Thus, initial communications to the public regarding exposure risks relied extensively on the epidemiology of previously identified highly pathogenic CoVs including SARS-CoV

type 1 [2] and Middle East respiratory syndrome (MERS-CoV) [3]. However, specific characterization of SARS-CoV-2 transmission pathways, including indirect contact via contaminated surfaces, and factors impacting exposure risk became crucial as the world reluctantly stepped into an unprecedented public health emergency.

Briefly, SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus within the genus *Betacoronavirus* of the family *Coronaviridae* [1]. The *Betacoronavirus* genus includes other highly pathogenic CoVs (i.e. SARS-CoV-1 and MERS-CoV). Replication of SARS-CoV-2 in both the respiratory and gastrointestinal tracts aligns with previous findings for SARS-CoV-1 [4]. Although SARS-CoV-2 manifests clinically as pneumonia in the lower respiratory tract of humans with some distinction across variants, significant and persistent viral loads are also detected in the upper respiratory tract and in stool samples of COVID-19 cases [5]. Characterization of both the concentration and route (i.e. fecal, respiratory) of viral shedding is of particular importance when investigating transmission pathways. More specifically, numerous environmental factors can influence *ex vivo* persistence of enveloped, respiratory viruses, such as SARS-CoV-2, on surfaces [6,7] which is the focus of the current review.

In this review, the authors discuss the current state of science regarding SARS-CoV-2 transmission via contaminated surfaces and persistence on environmental surfaces. In addition, the challenges and limitations of SARS-CoV-2 persistence research are discussed. Lastly, it is the opinion of the authors that guidelines (i.e. minimum information required) should be established for publication of virus persistence data for interpretation across studies and to facilitate the translation of research into practice. Overall, this review aims to provide the reader with an overview of the currently published persistence studies on SARS-CoV-2, the factors impacting persistence, guidelines for performing persistence studies, limitations of current data, and future directions for assessing SARS-CoV-2 persistence on fomites.

Severe acute respiratory syndrome-coronavirus-type 2 transmission pathways

The primary route of transmission for SARS-CoV-2 is via respiratory droplets (> 100 µm particles) and, to a lesser

extent, aerosols (< 100 µm particles) which are produced by coughing, sneezing, talking, and breathing [8,9]. Analysis of SARS-CoV-2 outbreak events has indicated a combination of both droplet and airborne transmission which is largely dependent on the specific setting (i.e. ventilation, ambient temperature, air flow) [10]. When considering infectious disease transmission via surfaces, respiratory droplets—as opposed to aerosols—are of concern since these larger expelled particles (i.e. a mixture of mucus, saliva, epithelial and immune cells, and, in this scenario, infectious virus) can deposit on proximal surfaces relatively quickly. This potential for particle deposition underscores the importance of characterizing the persistence of SARS-CoV-2 on surfaces, as subsequent transfer from contaminated hands to the mouth or mucosal membranes can result in infection.

While the significance of indirect contact transmission of SARS-CoV-2 via contaminated fomites is posited to be relatively minor, the ability to obtain direct evidence of any single transmission pathway is nearly impossible. Even still, numerous researchers have attempted to characterize or estimate the risk of SARS-CoV-2 transmission via fomites. Onakpoya et al. [11] conducted a systematic review to ascertain the role of fomites in SARS-CoV-2 transmission. After reviewing 63 primary studies along with one systematic review, the authors concluded that SARS-CoV-2 RNA can be frequently detected on fomites, yet no positive culture results were observed in studies that tested for virus infectivity. While the overall quality of the studies was low to moderate due to several factors including lack of descriptive methodology, appropriate analysis/reporting, and control of bias, these results suggest that RNA is more stable than infective SARS-CoV-2 virus [11]. Additionally, quantitative microbial risk assessments have been performed to model transmission risks [12,13], which further supports the notion of a low risk of SARS-CoV-2 transmission via fomites.

Severe acute respiratory syndrome-coronavirus-type 2 persistence on fomites

The persistence of SARS-CoV-2 on fomites has been observed for extended time periods (up to 21 days) across several research groups (Table 1). These studies were performed with an initial inoculum titer (on the surface) ranging from approximately 3 to 6 log TCID₅₀ or PFU. Unfortunately, previous studies may not accurately represent the actual virus titer on a ‘real-world’ surface. Many studies inoculate high inoculum titers as a worst-case scenario that are likely orders of magnitude higher than observed in public settings thus resulting in an overestimation of survival times [34,35]. Additional research is needed to delineate the impact of inoculum level on inactivation kinetics of SARS-CoV-2 [36,37]. However, it should be noted that Paton et al. [29] did

not observe differences in SARS-CoV-2 decay rates on stainless steel with low (8×10^3 PFU) versus high (8×10^5 PFU) inoculum levels. However, Paton and co-authors [29] did observe differences in virus survival based on surface type, with the longest survival on surgical mask material and stainless steel, and the shortest survival on a polyester shirt and bank notes. In addition, these authors [29] also highlighted the importance of detecting viable virus and not RNA levels, as RNA copy number reduced by 1-log over 21 days yet viable virus was unrecoverable after approximately 5 days on surfaces.

Liu et al. [28] observed prolonged survival (up to 7 days at room temperature) of SARS-CoV-2 on various surface types in contrast to previous studies with survival times of 3–4 days at room temperature [16,24]. These differences prompted Liu and coauthors to recommend the establishment of technical specifications to steer research on emerging viruses [28]. The authors of the current review agree that research groups should include specific information when designing and publishing studies on SARS-CoV-2 persistence on fomites (Table 2). Adherence to this minimum information required for publication will facilitate comparisons among currently available and future datasets. In addition, these guidelines will allow research groups to make more informed comparisons across studies, which is essential based on the numerous factors involved in persistence studies such as inoculum preparation/matrix, titer, recovery methods, temperature, relative humidity, among others (Table 1).

Temperature is an important factor in virus persistence. Typically, an increase in temperature results in a decrease in infectious virus. Numerous studies observed longer survival times for SARS-CoV-2 when assessing persistence at low versus high temperatures [18,22,27]. Conversely, Kratzel et al. [19] observed similar survival rates at 4 °C and room temperature as well as greater survival at 30°C. Notably, Kratzel et al. [19] investigated SARS-CoV-2 persistence with 0.3% bovine serum albumin (BSA) in the inoculum matrix. However, this does not explain the differences observed across temperatures as Riddell et al. [22] utilized a tripartite solution as an inoculum matrix, which also contains BSA along with mucin and tryptone. Riddell et al. [22] and Kratzel et al. [19] observed similar SARS-CoV-2 half-lives of 10.7–32.7 h and 17.9 h at 30°C, respectively. However, at room temperature, 40.3–65.8 h half-lives were observed by Riddell et al. [22] in tripartite solution and a half-life of 9.1 h in 0.3% BSA by Kratzel et al. [19]. Although differences in relative humidity (Riddell et al. [22]: 50%, Kratzel et al. [19]: 30–40%) may have contributed to the differences in half-lives observed between the two studies, these results highlight the complexity and variability within persistence studies across research groups.

Table 1
Summary of SARS-CoV-2 persistence studies on fomites.

Surface Type	Strain/Isolate	Inoculum titer (on surface)	Inoculum volume (μL)	Inoculum matrix	Recovery medium	Temperature ($^{\circ}\text{C}$)	Relative Humidity (%)	Survival time	Half life (h)	Reference
Glass and stainless steel	index case in Hong Kong	3×10^6 TCID ₅₀ ^a	5	DMEM with 2% FBS	VTM + 0.5% BSA + 0.1% glucose	22–23	60–70	> 1 day	–	[14]
stainless steel, plastic, nitrile gloves	USA-WA1/2020 (NR-52281)	–	1, 5, or 50	Simulated saliva	Complete growth media	24 35	20–80 20–60	–	8.33–15.33 2.26–7.52	[15]
Multiple surfaces	–	3.15×10^5 TCID ₅₀ ^a	5	–	VTM	22	65	0.5–96 h	–	[16]
Plastic, stainless steel, cardboard	–	2.5×10^3 to 5.0×10^3 PFU	50	Culture media	–	21–23	40	24–50 h	–	[17]
Swine skin, bank notes, clothing	USA-WA1/2020	3.2×10^4 PFU	50	EMEM with 10% FBS	MEM	4 22 37	40–50	96–336 h 4–96 h 4–8 h	15.9–46.8 1.0–3.5 0.2–0.6	[18]
Metal	SARS-CoV-2/München-1.1/2020/929	1.58×10^7 TCID ₅₀	–	0.3% BSA	Sterile water	4, RT, 30	30–40	–	9.1–17.9	[19]
Plastic	USA-WA1/2020	5×10^3 TCID ₅₀ ^a	50	human nasal mucus and sputum	–	4 21 27	40 40 85	–	3.3–5.8 3.1 1.5	[20]
Plastic, aluminum, glass	–	1×10^6 TCID ₅₀	50	Culture media	–	19–21	45–55	–	2.5 to > 96 > 96	[21]
Multiple surfaces	Betacoronavirus/Australia/SA01/2020	3.38×10^5 TCID ₅₀	10	3% BSA Tripartite solution	DMEM	20 30 40	50	–	40.3–65.8 10.7–32.7 1.4–3.0	[22]
Stainless steel	BetaCoV/France/IDF0571/2020	1.6×10^6 TCID ₅₀	50	Culture media	Culture media	7 25 7 25	65	96 h 72 h 48 h	–	[23]
Multiple surfaces	HCoV-19 n CoV-WA1–2020	5×10^3 TCID ₅₀ ^a	50	DMEM	DMEM	21–23	40	4–72 h	0.77–6.81	[24]
Stainless steel, glass, plastic	From symptomatic patient, Perugia, Italy	3.16×10^5 TCID ₅₀ ^a	10	MEM	MEM	23–25	40–50	72–120 h	4.2–5.3	[25]
Nitrile gloves, plastic, Tyvek, stainless steel	hCoV-19/Canada/ON-VIDO-01/2020	7.6×10^5 TCID ₅₀ ^a	10	Tripartite solution	DMEM + 2% FBS + 1% Pen-Strep	20	35–40	7–21 days	–	[26]
Multiple surfaces	USA-WA1/2020	5×10^4 TCID ₅₀	50	DMEM with 5% FBS	DMEM with 5% FBS	5 13 25 21	75 66 70 60	Up to 21 days Up to 7 days Up to 4 days < 3 days	67.21–121.78 17.11–31.82 2.99–5.58 3.5–12.86	[27]
Multiple surfaces	BetaCoV/Beijing/AMMS01/2020	5×10^4 TCID ₅₀ ^a	50	–	VTM	RT	–	Up to 7 days	–	[28]
Multiple surfaces	England -2/2020 (EPI_ISL_407073)	8×10^3 to 8×10^5 PFU ^a	20 (two 10 μL droplets)	cMEM	cMEM	21.5	45	< 1–14 days	–	[29]
Multiple surfaces	USA-WA1/2020	1×10^4 PFU	50 (spread)	–	Cell culture media	25	45–50	< 4–48 h	–	[30]

Table 1 (continued)

Surface Type	Strain/Isolate	Inoculum titer (on surface)	Inoculum volume (μL)	Inoculum matrix	Recovery medium	Temperature ($^{\circ}\text{C}$)	Relative Humidity (%)	Survival time	Half life (h)	Reference
Pelts, faux leather, polyester, plastic Three types of paper Banknotes, credit card, quarter, penny	SARS-CoV-2/Finland/1/2020	5×10^4 PFU ^a	50 (spread)	MEM with 2% FBS	MEM with 2% FBS	RT	40–75	< 30 min to 10 days	-	[31]
	JPN/TY/WK-521	2×10^5 FFU or TCID ₅₀	2	DMEM	DMEM	25	40–50	6.48–59.78 h	0.22–4.06	[32]
	2019-nCoV/USA-WA1/2020	3×10^5 PFU ^a	20	DMEM	DMEM	22	-	4–48 h	-	[33]

^a Calculated based on inoculum volume; FFU — focus-forming units; DMEM — Dulbecco Modified Eagle's Medium; MEM — Eagle's minimum essential medium; cMEM — complete minimal essential medium; VTM — viral transport medium; Tripartite solution consists of mucin, bovine serum albumin, and tryptone

When considering the surface type (e.g. plastic, stainless steel, etc.), there seem to be no major differences in SARS-CoV-2 survival among different types of non-porous surfaces [15]. However, when comparing non-porous surfaces with porous surfaces (e.g. vinyl, cotton, paper, polyester, etc.), much shorter SARS-CoV-2 survival times are observed on porous surfaces [16,29,30,38]. Chatterjee et al. [39] highlighted the importance of surface wettability and the impact on thin-film evaporation and subsequent virus survival. Once the initial evaporation of a bulk droplet occurs on a surface, the evaporation of the remaining residual thin film is driven by disjoining pressure within the film. Thus, the wettability and overall surface topography can impact survival based on the integrity of the thin film [40]. Identifying which factors impact surface wettability, such as frequency of use and the presence of organic matter residues following cleaning, may help predict the variability in persistence on different surfaces.

Surface inoculation procedures can also impact the observed survival of SARS-CoV-2. For instance, virus infectivity is greatly reduced during the initial drying process [19], after which the dried inoculum can survive and result in transfer. Biryukov et al. [15] did not observe significant differences in half-lives impacted by inoculum volumes of 5, 10, or 50 μL . A majority of studies on SARS-CoV-2 persistence were evaluated with cell culture media (e.g. minimum essential medium) as the inoculum matrix; however, determining SARS-CoV-2 persistence within matrices that are more representative of real-world scenarios (i.e. respiratory secretions, fecal shedding) is critical to the relevance of data to the food industry and beyond. For instance, Liu et al. [28] observed that SARS-CoV-2 can survive for several hours in feces and several days in urine; thus, identifying how the virus survives on surfaces with accompanying matrices in the environment should be an important consideration when designing future persistence studies. In the context of transmission routes via fomites in food-related environments, simulated saliva and human nasal mucus and sputum are likely the most representative of the matrix accompanying infectious virus on fomites [15,20,23]. However, the ability of infectious virus shedding via feces could also be of particular importance to understanding indirect contact transmission in food-related environments [5,41].

Lack of evidence for foodborne transmission

Strategies such as handwashing and not working while ill are well known in the food industry for preventing infectious disease transmission; however, time constraints and a lack of food safety culture can greatly impact how well these strategies are implemented, thus impacting the risk of pathogen transmission. Evidence supporting the foodborne transmission of SARS-CoV-2 is lacking

Table 2**Critical, preferred, and beneficial information to provide for SARS-CoV-2 persistence studies with example references in brackets.**

Critical	Preferred	Beneficial
Inoculum application (spread versus droplet) [29–31] Inoculum matrix [15,22,26]	Calculated half-life values	Inoculum matrix characterization [15]
Inoculum titer [19,22,27,30] Inoculum volume Relative humidity Recovery method (repeated pipetting, flooding) [22,29] Sampling time points Strain/isolate type Temperature	Continuous monitoring of temperature and relative humidity Limit of detection Source and details of surface(s) [30]	Inoculum temperature

[42]. Current practices among food industry workers and management that target highly transmissible foodborne pathogens such as human norovirus [43] will lower the risk of SARS-CoV-2 transmission via food even further. The Food and Agriculture Organization of the United Nations has provided guidance for preventing COVID-19 transmission within food businesses and suggests establishments to adhere to all applicable food hygiene standards and other preventive measures currently in place [44].

Given that SARS-CoV-2 is a respiratory virus, it is critical to acknowledge that the greatest risk to the food industry is transmission among workers and not the consumption of foods that may have been handled with SARS-CoV-2 contaminated hands or surfaces. The potential for SARS-CoV-2 transmission among workers during meat processing has been documented [45], and fomites have been suspected of indirect transmission SARS-CoV-2 [46]. Liu et al. [47] also described the possibility of SARS-CoV-2 transmission via surface contamination of the outer packaging of imported frozen cod after two port personnel were determined to be positive for SARS-CoV-2. Chi et al. [48] further outlined instances of SARS-CoV-2 detection on cold chain food packaging though evidence of subsequent contact transmission was decidedly inconclusive. While the occurrence of SARS-CoV-2 on frozen food packaging is compelling, simulation models suggest that additional decontamination processes on food packaging materials for control of SARS-CoV-2 do not confer beneficial risk reduction for cold-chain food workers when combined with effective practices such as handwashing and mask-wearing [49]. Handwashing and mask-wearing were shown to reduce risk well below the risk threshold, thus decontaminated packaging was suggested to have minimal impact on risk reduction. While the COVID-19 pandemic may impact food safety in the food industry due to supply chain disruptions, disruptions in food safety practices in food processing facilities, and

disruptions in audits, the risk of SARS-CoV-2 transmission via food is negligible [50].

Jai et al. [51] observed infectious SARS-CoV-2 for up to 21 days post inoculation (initial titer of 4 log PFU) on refrigerated deli foods, meats, and fresh produce, although the risk of foods having a high enough virus concentration to result in transmission is unlikely. Fortunately, most foods are treated using various processes (e.g. acidification, heat treatment, etc.) to greatly reduce the risks of biological hazards. For example, both acidification and heat treatment have shown to impact the nucleocapsid of SARS-CoV-1 [52]. If food and/or its ingredients became contaminated with SARS-CoV-2, routinely applied food processing steps would greatly reduce the risk of transmission via food [53].

Challenges and limitations of severe acute respiratory syndrome-coronavirus-type 2 persistence studies and future directions

Although further investigations are warranted, Hirose et al. [54] observed longer survival of the SARS-CoV-2 Omicron variant on human skin surfaces in comparison with the Wuhan, Beta, Gamma, and Delta variants. The survival among SARS-CoV-2 variants of concern may be of interest in future persistence studies on porous and nonporous surfaces. The work by Hirose et al. [54] further highlights the importance of not generalizing virus persistence on fomites as this is a long withstanding route of transmission, and not all viruses behave similarly on environmental surfaces. These differences between variants may be due in part to structural changes of the viral capsid allowing for increased resistance to environmental stressors [4]. A study evaluating the persistence of SARS-CoV-2 variants would be beneficial for characterizing how variant type impacts SARS-CoV-2 persistence. Additionally, assuming that differences similar to Hirose et al. [54] will be observed, further evaluation of the structural components of each variant and corresponding survival rates would greatly aid in the

understanding of future variants or emerging virus persistence on fomites.

Optimizing real-world scenarios that include more representative surface conditions observed in public settings should be investigated. For example, several studies utilized an artificial organic load matrix, that may differ from actual inoculum composition to a greater extent than realized in public settings. Additionally, grease, food residues, and other components on surfaces that are not removed before contacting the virus may need to be further characterized to fully understand virus interactions with surfaces in a more realistic environment. The microbiota present on fomites should not be ignored as these microorganisms likely interact with SARS-CoV-2 [55] which possibly impacts virus inactivation kinetics. While these biological questions are of importance, researchers continuously balance time and resources during their efforts to address critical knowledge gaps. Preliminary studies for experimental design and optimization with SARS-CoV-2 are limited due to BSL-III facility requirements, as well as the time and troubleshooting that may be associated with confirming the absence of cytotoxic effects of the inoculum or surface matrices on host cell lines.

Conclusion

This review provides an overview of the current available data on SARS-CoV-2 persistence on fomites in the context of consumer-facing environments including food-related settings. Based on the current state of science regarding SARS-CoV-2 transmission via contaminated surfaces and its persistence on environmental surfaces, there is a relatively low risk of fomite transmission, and mitigation efforts for future emerging viruses should emphasize proper hygienic practices, physical distancing, and proper air ventilation in food-related environments. Nevertheless, some transmission via fomites likely occurs, although delineating transfer rates through validated studies is an extremely difficult task. Overall, future research on SARS-CoV-2 persistence on fomites should address issues pertaining to the inoculum titer, matrix, and variants used in survival studies. Lastly, adhering to the guidelines on the minimum information required for publishing is recommended to guide future researchers and alleviate the difficulty of comparing persistence data across studies with contrasting variables.

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- of special interest
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