Transfer Rate of Enveloped and Nonenveloped Viruses between Fingerpads and Surfaces

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ABSTRACT Fomites can represent a reservoir for pathogens, which may be subsequently transferred from surfaces to skin. In this study, we aim to understand how different factors (including virus type, surface type, time since last hand wash, and direction of transfer) affect virus transfer rates, defined as the fraction of virus transferred, between fingerpads and fomites. To determine this, 360 transfer events were performed with 20 volunteers using Phi6 (a surrogate for enveloped viruses), MS2 (a surrogate for nonenveloped viruses), and three clean surfaces (stainless steel, painted wood, and plastic). Considering all transfer events (all surfaces and both transfer directions combined), the mean transfer rates of Phi6 and MS2 were 0.17 and 0.26, respectively. Transfer of MS2 was significantly higher than that of Phi6 ($P < 0.05$). Surface type was a significant factor that affected the transfer rate of Phi6: Phi6 is more easily transferred to and from stainless steel and plastic than to and from painted wood. Direction of transfer was a significant factor affecting MS2 transfer rates: MS2 is more easily transferred from surfaces to fingerpads than from fingerpads to surfaces. Data from these virus transfer events, and subsequent transfer rate distributions, provide information that can be used to refine quantitative microbial risk assessments. This study provides a large-scale data set of transfer events with a surrogate for enveloped viruses, which extends the reach of the study to the role of fomites in the transmission of human enveloped viruses like influenza and SARS-CoV-2.

IMPORTANCE This study created a large-scale data set for the transfer of enveloped viruses between skin and surfaces. The data set produced by this study provides information on modeling the distribution of enveloped and nonenveloped virus transfer rates, which can aid in the implementation of risk assessment models in the future. Additionally, enveloped and nonenveloped viruses were applied to experimental surfaces in an equivalent matrix to avoid matrix effects, so results between different viral species can be directly compared without confounding effects of different matrices. Our results indicating how virus type, surface type, time since last hand wash, and direction of transfer affect virus transfer rates can be used in decision-making processes to lower the risk of viral infection from transmission through fomites.

KEYWORDS virus transfer, fomites, surfaces, hand hygiene, bacteriophages, MS2, Phi6, enveloped virus, fomite, transfer, virus

Viruses are deposited in the environment when fluids (such as mucus, saliva, urine, and feces) containing high viral titers are released from an infected individual (1, 2). Humans can come into contact with viruses when they consume or recreate in virus-contaminated water, eat contaminated food, breathe contaminated air, or touch contaminated fomites. When transmission of a virus occurs via an environmental intermediary such as air, water, or fomite, the transmission is referred to as "indirect." It is well understood that indirect transmission is important for many viruses, including those that cause diarrheal illness, influenza, COVID-19, and measles (2–8). While it is well known that fomite-mediated transmission is an important
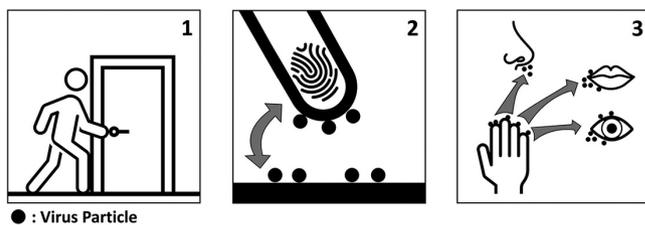
● : Virus Particle

FIG 1 Pathway of transmission from contaminated fomites. First, the individual contacts a surface from which the individual picks up or deposits invective virus particles. Finally, the individual transfers the infective virus from their hand to an area of their body, where infection occurs, or to an additional surface.

pathway for many diseases, several studies have emphasized the need for more information about inactivation rates, transfer rates, and pathogen shedding to develop accurate exposure and risk models (2, 3, 6, 9, 10).

Transmission of viruses via contaminated fomites requires multiple steps (Fig. 1). First, a susceptible individual must come into contact with the surface. Second, viruses are transferred between the fomite and the susceptible individual. Third, the virus transferred via touch is transmitted to the individual. The last step may require an additional transfer event from the part of the body that touched the fomite to another part of the body where infection occurs (sometimes referred to as self-inoculation). Whether the transmission event results in infection depends on the biology of the virus and the immune system of the individual. Infected individuals can also deposit viruses onto fomites via touch if there is virus present on their body, thereby contaminating fomites with viruses. In the present study, we are particularly focused on the transfer of viruses to and from skin and fomites.

Six studies have characterized the transfer of viruses to and from skin and inanimate surfaces, and they have been been undertaken primarily using nonenveloped viruses (Table 1) (1, 5, 11–14). This collection of studies includes virus transfer studies that explicitly quantified transfer rates between human skin and nonfood surfaces (15) (see Table 1 of Zhao et al. [15] for a complete list of all virus transfer studies). These studies quantified transfer of MS2 (1, 11), poliovirus 1 (1), human parainfluenza virus (5), rhinovirus (5, 14), ϕX174 (11), fr (11), rotavirus (12), and hepatitis A (13). Of these viruses, only human parainfluenza is enveloped. When investigating human parainfluenza virus transfer between skin and surfaces, Ansari et al. (5) found that the virus inactivated quickly; therefore, it was impossible to quantify the transfer rate. Although experimental variables such as humidity, surface type, and virus type vary for each study, aspects of the experimental procedures remain relatively consistent for each study. Four of the six studies (1, 5, 12, 13) have an inoculation volume of 10 μl, a contact time of 5 to 10 s, 20 to 30 min of inoculum dry time, and a contact pressure of 1 kg/cm². All 6 studies quantify virus transfer rate, which is defined as the fraction of virus transferred upon contact. Julian et al. (11), who used methods similar to those used here (Fig. 2), define transfer rate as the number of PFU recovered from the noninoculated surface over the total number of PFU recovered from both surfaces. One of the studies investigated transfer rates between both porous and nonporous fomites (1), while the rest studied only nonporous fomites. Across all 6 studies, transfer rate varied between <0.02 and 0.80 for nonporous surfaces (1, 5, 11–14). The study investigating porous surfaces found a transfer rate of <0.07 (1).

Zhao et al. (15) provide a mechanistic model of transfer rates between surfaces. Their model considers the physical and chemical mechanisms that control transfer. Their model suggests that touch force, microbial diameter, inoculation volume, touch number by the same individual, rubbing, and humidity have a positive correlation with virus transfer. They also suggest that donor roughness, touch number by different individuals, surface hardness, temperature, surface inoculation area, and surface touching area are negatively correlated with virus transfer.

TABLE 1 Summary of previous studies investigating viral transfer rates[a]

Reference or source	Virus species	Surface(s)	Inoculation vol, in μl (concn)	Contact time (s)	Inoculum dry time (min)	Contact pressure	Transfer rate
Reed (14)	Rhinovirus	Plastic pen, table, stainless steel	2–5.5 (NA)	15	2–10	NA	Mean up to 0.46
Ansari et al. (12)	Rotavirus	Stainless steel	10 (NA)	10	20	1 kg/cm^2	Mean up to 16.8
Ansari et al. (5)	Human parainfluenza virus 3, rhinovirus 14	Stainless steel	10 (NA)	5	20	1.0 kg/cm^2	Mean up to 0.02
Mbithi et al. (13)	Hepatitis A	Stainless steel	10 (NA)	10	20	0.2 kg/cm^2 to 1 kg/cm^2	Mean up to 0.27
Julian et al. (11)	MS2, ϕX174, fr	Glass	5 ($\sim$10^6 PFU/ml for all viruses)	10	10	0.25 kg/cm^2	Mean of 0.23
Lopez et al. (1)	MS2, poliovirus 1	Acrylic, glass, ceramic tile, laminate, stainless steel, granite, cotton, polyester, paper currency	10 ($\sim$10^{10} PFU/cm^2 MS2 and $\sim$10^8 PFU/cm^2 poliovirus 1)	10	30	1.0 kg/cm^2	Nonporous surfaces, mean up to 0.80; porous surfaces, mean of <0.07
This study	MS2 Phi6	Plastic, stainless steel, painted wood	10 ($\sim$10^5 PFU/ml MS2 and $\sim$10^9 PFU/ml Phi6)	10	Up to 30	0.25 kg/cm^2	Mean up to 0.26

[a]Included are various experimental variables, including virus species, inanimate surface, inoculation volume (and concentration, if available), contact time, inoculum dry time, and contact pressure. NA, not available.
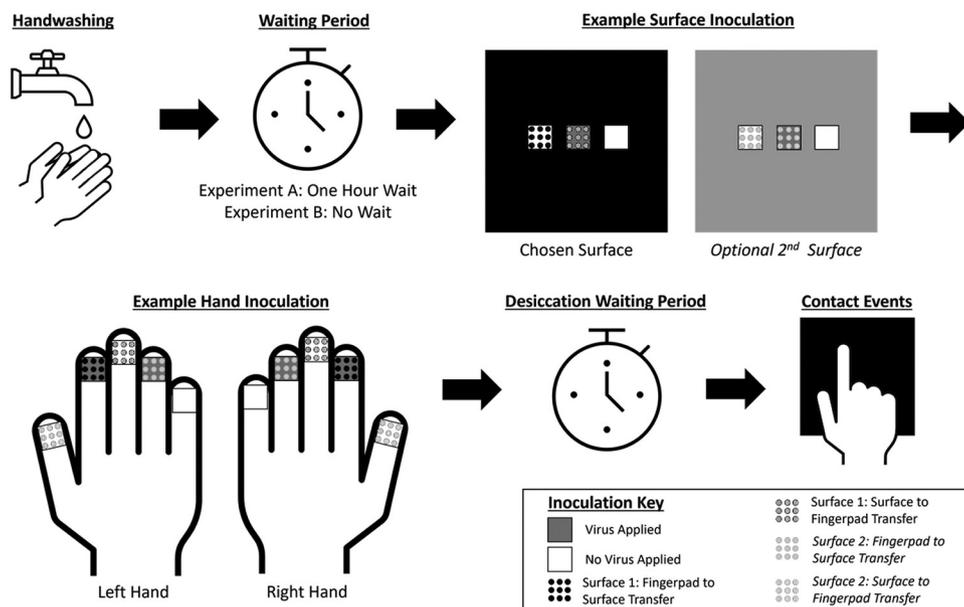
FIG 2 Outline of experimental procedure up until the contact events. The procedure outlines the initial hand-washing step, followed by the wait time for experiment A or B, an example of surface inoculation, an example of hand inoculation, the wait time for the inoculum to dry, and the contact events. In the example surface inoculation, the leftmost square represents where the virus was not applied but where the transfer from the fingerpad to the surface would occur. The middle square represents where the virus was applied (indicating transfer from the surface to the fingerpad), and the rightmost is the control. In the example hand inoculation, the hands are duplicates. On the middle fingerpad and thumb there is no virus applied, but it represents where the transfer from the chosen surface and 2nd surface to the fingerpad will occur. Virus is applied on the index fingerpad and ring fingerpad, where transfer between the fingerpad and the respective surfaces will occur. The pinky is a control.

There is presently no experimental data on transfer of enveloped viruses between skin and surfaces, so this study sought to fill that knowledge gap. We documented the transfer rate of enveloped and nonenveloped viruses between various surfaces and fingerpads using human volunteers and 360 transfer events, creating a large-scale data set for enveloped viruses. The data set produced by this study provides information on modeling the distribution of enveloped and nonenveloped virus transfer rates, which can aid in the implementation of risk assessment models in the future (9, 16–18).

We also investigated how virus type, surface type, time since last hand wash, and direction of transfer affect virus transfer rates. The choice of variables is informed by results of previous studies and the model developed by Zhao et al. (15). Enveloped and nonenveloped viruses were applied to experimental surfaces in an equivalent matrix to avoid matrix effects, so the results obtained with different viral species can be directly compared without confounding effects of different matrices.

The enveloped virus used in this study is Phi6. Phi6 has a double-stranded RNA genome and is spherical in shape, with an ~80- to 100-nm diameter; the protein nucleocapsid is surrounded by a lipid membrane and, thus, serves as a nonpathogenic, biosafety level 1 bacteriophage surrogate for enveloped human-pathogenic viruses, such as influenza, SARS-CoV-2, and Ebola. The nonenveloped virus used in this study is MS2. MS2 has an single-stranded RNA genome and an icosahedral protein shell ~27 nm in diameter. MS2 similarly acts as a biosafety level 1 bacteriophage surrogate for nonenveloped human-pathogenic viruses such as norovirus and enteroviruses. Phi6 and MS2 were previously applied to hands to model pathogenic viruses (11, 19–21).

RESULTS

Experimental conditions. A total of 20 volunteers participated in the study. They ranged in age from 22 to 58 years, with the median age being 26. Volunteer hand

length ranged from 16.2 cm to 21.9 cm, with the median length being 19.3 cm. Volunteer hand breadth ranged from 7.3 cm to 10 cm, with a median breadth of 8.1 cm. Temperature of the laboratory throughout the study ranged from 20.8°C to 21.9°C, with a median temperature of 21.7°C. Relative humidity (RH) during the study ranged from 13% to 74%, with a median value of 58%. As the building has a passive cooling system, RH indoors reflects outdoor RH and is not controlled. Full temperature and humidity data are available in the supplemental material.

Transfer rate distributions. All negative controls had 0 PFU, and positive controls of all viral stock concentrations had consistently high virus concentrations, confirming the assays worked (10^{10} to 10^{11} PFU/ml for both Phi6 and MS2).

The fraction of virus transferred (f) was determined for 360 transfer events for the two viruses. Out of the 360 transfer events for Phi6, all dilutions plated were TNTC (too numerous to count) 8 times. No PFU were detected on the undiluted sample plate 38 times. These 46 Phi6 transfer events were removed from the data set given the uncertainty of their true value, leaving 314. Out of the 360 transfer events for MS2, there were no instances where all dilutions were TNTC. No PFU were detected 4 times for MS2. As a result, 4 MS2 transfer events were removed from the data set for MS2, leaving 356. These instances where the transfer rate was irrecoverable for Phi6 and MS2 are not limited to a single surface, time since last hand wash, or direction of transfer. The instances also make up less than 7% of the total data and, therefore, are not anticipated to affect the overall distribution of the data. More information about these instances of irrecoverable transfer rates can be found in Table 2 and the supplemental material.

The mean transfer rate for Phi6 was 0.17, while the median was 0.12 and the standard deviation was 0.17. For MS2, the mean transfer rate was 0.26, the median was 0.25, and the standard deviation was 0.18 (Fig. 3 and 4). The respective means, medians, and standard deviations of the transfer rate based on the variables investigated (virus type, surface type, time since last hand wash, and direction of transfer) can be found in Table 2 and Fig. 3. Note that these results, and others described below, are stable under a range of different assumptions regarding samples that were TNTC or below the detection limit (see the supplemental material).

Several distributions (including normal, lognormal, exponential, geometric, and beta) were fit to the data, and the goodness of fit for each was tested through a Kolmogorov-Smirnov test, comparing the log likelihood, and comparing the Akaike's information criterion (AIC). Overlayed on the histogram in Fig. 4 is the distribution that best fit the data, along with the distribution parameters. In the case of both virus types, beta distributions fit the data best. For each virus, the beta distribution had the highest log-likelihood estimate, the lowest AIC, and a P value greater than 0.05. Although the normal distribution fit the data well (P values of 0.46 and 0.54 for Phi6 and MS2, respectively), it was not used because it included the possibility of negative transfer fraction values, which are physically unrealistic.

Significant factors controlling transfer rate. An n-way analysis of variance (ANOVA) on the complete data set indicates that virus type ($P < 0.001$), surface type ($P < 0.001$), and direction of transfer ($P < 0.001$) are significant factors in controlling transfer. An ANOVA is justified for analyzing these data, as the Kolmogov-Smirnoff test suggested the data could be reasonably approximated as normal. No blocking or cluster analysis was used in the ANOVA, based on a more preferable AIC without blocking than with. Blocks we tested include those for the fingerpad (thumb, index, middle, ring, or pinky), hand (left versus right), and volunteer. The time since last hand wash factor was not significant in the model ($P = 0.87$). In terms of interactions between variables, significant two-way interactions were found between the virus type and surface type, the virus type and time since last hand wash, and surface type and time since last hand wash. The remaining unlisted interactions were not statistically significant. To parse these interaction terms, two three-way ANOVAs were performed with Phi6 and MS2 as the dependent variables, separately.

A three-way ANOVA performed with Phi6 transfer rate as the dependent variable

TABLE 2 Descriptive statistics for the transfer rates of Phi6 and MS2[a]

Virus and surface	Time since last hand wash (h)	Direction of transfer	No. of trials	Transfer rate		
				Mean	Median	SD
Phi6						
Stainless steel	1	Surface to fingerpad	30	0.23	0.19	0.19
		Fingerpad to surface	25	0.18	0.16	0.20
	0	Surface to fingerpad	30	0.20	0.17	0.15
		Fingerpad to surface	22	0.22	0.21	0.15
Plastic	1	Surface to fingerpad	30	0.28	0.22	0.23
		Fingerpad to surface	22	0.17	0.09	0.19
	0	Surface to fingerpad	30	0.22	0.21	0.14
		Fingerpad to surface	22	0.15	0.11	0.12
Wood	1	Surface to fingerpad	28	0.05	0.01	0.07
		Fingerpad to surface	26	0.13	0.09	0.14
	0	Surface to fingerpad	27	0.08	0.03	0.10
		Fingerpad to surface	22	0.07	0.05	0.06
MS2						
Stainless steel	1	Surface to fingerpad	30	0.34	0.33	0.12
		Fingerpad to surface	30	0.13	0.08	0.12
	0	Surface to fingerpad	30	0.37	0.37	0.12
		Fingerpad to surface	30	0.18	0.13	0.17
Plastic	1	Surface to fingerpad	30	0.37	0.33	0.14
		Fingerpad to surface	30	0.16	0.11	0.16
	0	Surface to fingerpad	30	0.40	0.37	0.18
		Fingerpad to surface	29	0.15	0.11	0.17
Wood	1	Surface to fingerpad	30	0.30	0.29	0.18
		Fingerpad to surface	28	0.22	0.17	0.17
	0	Surface to fingerpad	30	0.33	0.29	0.20
		Fingerpad to surface	29	0.21	0.19	0.18

[a]Statistics are broken down by virus type, surface, time since last hand wash, and direction of transfer. Included in the statistics are the number of trials for each condition as well as the mean, the median, and the standard deviation of the transfer rate.

indicates that surface type is significant ($P < 0.001$). The *post hoc* test shows that there are differences between wood and plastic (mean difference between wood and plastic, -0.13; $P < 0.001$) and wood and stainless steel (mean difference between wood and stainless steel, -0.12; $P < 0.001$) but no difference between stainless steel and plastic
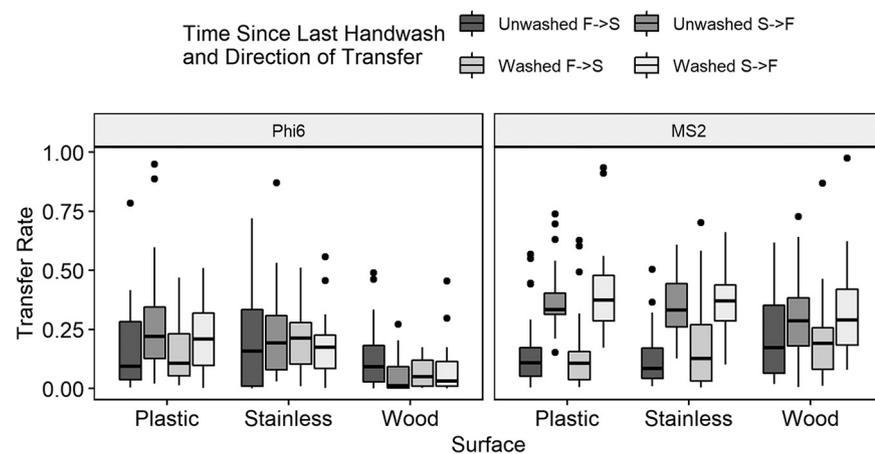

FIG 3 Boxplots of transfer rates for different surfaces. The upper and lower whiskers show the maximum and minimum values, respectively (excluding outliers defined by the interquartile range criterion). The lower and upper edges of the box represent the lower and upper quartile, respectively. The horizontal line within the box indicates the median. The points beyond the whiskers represent outliers. The data are broken down by virus type, surface type, time since last hand wash, and direction of transfer. Unwashed represents 1 h since last hand wash, and washed represents 0 h since last hand wash. F->S represents fingerpad-to-surface transfer, and S->F represents surface-to-fingerpad transfer.
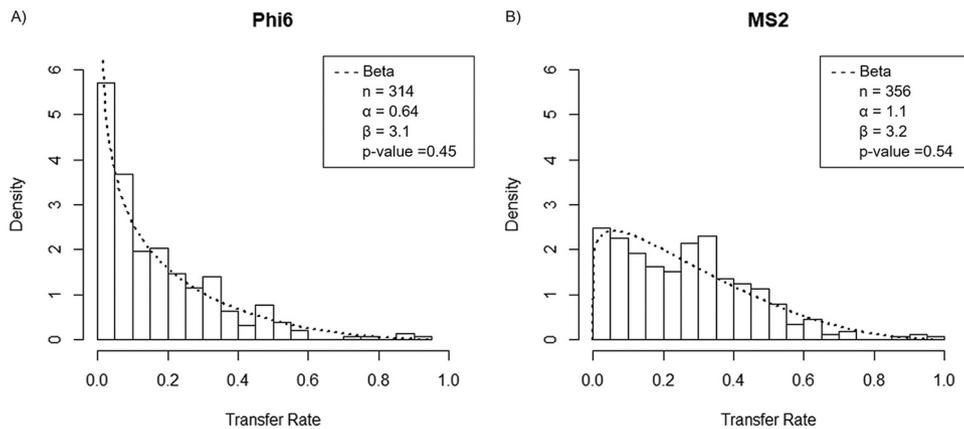
FIG 4 Phi6 (A) and MS2 (B) histogram distributions, overlaid with probability distribution functions. The *y* axis shows the density or number of observations for the transfer values. The functions used to model the data are beta distributions. The alpha and beta shape parameters, as well as the goodness-of-fit *P* values, are also shown.

($P = 0.97$). Direction of transfer ($P = 0.16$) and time since last hand wash ($P = 0.24$) are not significant factors in the model. There is no statistically significant three-way interaction between surface type, direction of transfer, or time since last hand wash ($P = 0.14$). In terms of possible two-way interactions, the only significant interaction occurs between surface type and direction of transfer ($P = 0.014$); the direction of transfer was found to significantly impact only the transfer rate between fingerpads and plastic (mean difference between fingerpad to plastic transfer and plastic to fingerpad transfer, -0.09).

A separate three-way ANOVA performed for all MS2 data indicates that direction of transfer is the only significant variable ($P < 0.001$). The *post hoc* test shows that the mean difference between fingerpad-to-surface transfer and surface-to-fingerpad transfer is -0.18. Surface type ($P = 0.71$) and time since last hand wash ($P = 0.23$) were not found to be significant. Similar to Phi6, there is no statistically significant three-way interaction between surface type, direction of transfer, or time since last hand wash ($P = 0.73$). The only significant two-way interaction occurs between the surface type and direction of transfer ($P = 0.003$). The direction of transfer significantly affects the transfer from all three surfaces, with a higher fraction transferred from surfaces to fingerpads for all surface types (a mean difference of 0.23 for plastic, 0.21 for stainless steel, and 0.10 for wood).

DISCUSSION

Both enveloped and nonenveloped viruses are readily transferred between fomites and fingerpads, with transfer rates of 0.22, on average. This implies that a transfer of 22% of viruses on a surface to a fingerpad should be expected. Whether or not this transfer would result in the risk of fomite-mediated infection would depend on the number of infectious viruses contacted by the fingerpad, the efficiency of self-inoculation (i.e., transfer of virus from fingerpad to the mouth, nasal cavity, eyes, or other bodily location where infection may occur), the infectious dose of the virus, and the susceptibility of the individual.

The transfer rates reported in this study for MS2 and Phi6 are similar to virus transfers reported by others (1, 12, 13, 22). Specifically, the MS2 mean transfer rate of 0.26 is comparable to the MS2 mean transfer rate of 0.22 between fingerpads and glass reported by Julian et al. (11), who used methods similar to those used here. Previous work reported viral transfer rates between skin and fomites to range between 0.16 and 0.65 for nonporous surfaces (1, 11–13, 22). The higher values in this range were obtained using greater contact pressure and a shorter desiccation time for viral

suspensions (1, 11, 13). According to a physical-chemical model of skin-surface micro-bial transfer (15), greater contact pressure will likely lead to higher transfers. Future work should explore the influence of this variable on viruses, and specifically nonenvel-oped viruses, experimentally.

Enveloped virus Phi6 is transferred between surfaces and fingerpads to a lesser extent than nonenveloped virus MS2. This suggests that enveloped viruses are trans-ferred less efficiently than nonenveloped viruses; however, the effect size is small (dif-ference in mean transfer rate is ~0.1). Both experimental and modeling studies sug-gest that enveloped and nonenveloped viruses can be transmitted via fomites and that this transmission requires transfer via a contact event and subsequent self-inocu-lation. For example, nonenveloped norovirus was shown experimentally in a case study to be transmitted via contaminated surfaces in a houseboat used by different groups in series (23). *Betaarterivirus suid 1*, an enveloped virus that infects pigs, was shown experimentally to be transmitted via contaminated fomites in a controlled animal ex-posure study (24). Results from Zhao et al. (15) indicate fomites can be important in the spread of enveloped influenza viruses. Boone and Gerba (2) summarize evidence on the role of fomite-mediated transmission of both enveloped and nonenveloped viruses from experimental studies and conclude its role can be important for both types of viruses. It will be important to repeat our study with a broader range of envel-oped viruses to confirm the reduced transferability of enveloped versus nonenveloped viruses.

Enveloped virus transfer is higher from smooth plastic and metal surfaces than rough wooden surfaces. Although stainless steel, plastic, and wood are all considered nonporous surfaces, the surface of painted wood is inherently more irregular due to brush strokes. This suggests that the microvariations in the surface of the wood create a less efficient transfer and, therefore, a lower transfer rate of the virus. Such heteroge-neities on the surface may prevent efficient contact between fingerpads and the surfa-ces. Previous studies have modeled that as donor roughness increases, the transfer rate decreases, based on touch probability and adhesive probability (15). However, as recipient roughness increases, the transfer rate correlation is nonmonotonic (15).

Nonenveloped viruses are more readily transferred from surfaces to fingerpads than from fingerpads to surfaces; the mean difference between surface to fingerpad and fin-gerpad to surface transfer rate was found to be 0.23 for plastic, 0.21 for stainless steel, and 0.10 for wood. In previous studies that report that direction of transfer is important in controlling virus transfer, conclusions regarding the direction in which virus was more readily transferred differed based on virus type (5, 11, 13). This agrees with what was found in this study, where only MS2 showed a greater transfer from surfaces to fin-gerpads than from fingerpads to surfaces. A greater transfer from surfaces to finger-pads than from fingerpads to surfaces suggests individuals can pick up viral particles from a surface and may not be able to spread them to additional surfaces as easily. As a result, viruses may remain on the skin rather than be transferred off. The presence of viruses on the hands and subsequent interaction with the nose, eyes, or mouth may lead to self-inoculation and subsequent infection. A previous study found that the transfer rate for a nonenveloped virus (PRD-1) from fingerpad to lip is roughly 0.34 (22). Additional work investigating skin-to-skin transfer rate, in combination with previ-ous results of surface-to-skin transfer rate, can help develop a complete model of the disease transmission pathway.

We did not find that time since last hand wash affected transfer of virus between surfaces and fingerpads. In general, handwashing can change the physiochemical properties of the skin, including changing the pH, removing dirt or oil, or leaving behind trace soap chemicals (25). A previous study found that recently washed hands led to decreased transfer of nonenveloped viruses to and from fingerpads and glass and speculated this was a result of changes in moisture level, pH of skin, and other re-sidual effects from the soap (11). Future work that investigates the effects of hand washing under different realistic scenarios, for example, with hands that are unwashed

for longer periods of time after work outdoors or shopping, may provide additional insights into the extent to which handwashing reduces or facilitates virus transfer between fingerpads and surfaces. Studies to examine the effect of different handwashing approaches on viral transfer, including the use of alcohol-based hand sanitizer (ABHS) would be interesting.

There are several limitations to this study that have not already been mentioned. First, this study controlled contact pressure even though it is understood that this may affect transfer (13, 15). Additional work to include contact pressure as a variable may be useful. Second, this study worked with clean surfaces and relatively clean fingerpads. In reality, surfaces and fingerpads may be coated with dirt or oil, and this could affect transfer rates by changing physiochemical interactions between viruses and surfaces (15). Further work should consider the use of realistically soiled surfaces and hands, which may provide protection to pathogens when the contact event occurs (26). Third, this study was restricted to two viruses and three surfaces. It would be interesting to expand on these in future studies to investigate whether the trends observed here for enveloped viruses can be confirmed with other surrogate, nonpathogenic enveloped viruses. Our surface sampling technique may not recover all viruses from the surfaces swabbed. An inherent assumption in this work is that the recovery efficiency of virus from fingerpads and tested surfaces was not distinct, so that the transfer rate could be calculated without accounting for recovery efficiency (as recovery efficiency would cancel out the numerator and denominator of equation 1). Recent work attempts to more accurately represent bacterial concentrations on surfaces using a sequential sampling method (15, 27). Future work should investigate the usefulness of this method for viruses and how its use might affect the calculation of transfer efficiencies. An additional limitation is that we had to seed surfaces with higher concentrations of Phi6 than MS2 to measure transfer using our approaches; this suggests that Phi6 inactivates when it is initially seeded on the surfaces under the conditions tested or that some Phi6 is not recoverable from the surfaces. While this study was not designed to study inactivation of enveloped viruses, additional work is needed to better understand persistence of enveloped viruses on surfaces (28). Finally, due to a relatively small volunteer number, we were unable to aggregate the data based on volunteer characteristics to test for the effects of gender, age, or race on the transfer rate. Volunteer characteristics were not used as a variable to predict virus transfer in the model created by Zhao et al. (15) but may influence variables that have been used to predict virus transfer. For example, males typically have larger hand sizes than females, which leads to increased surface touching area, which could lead to decreased transfer (15, 29). These associations can be studied in the future with a larger volunteer population.

This study provides a large-scale data set of transfer events with a surrogate for enveloped viruses, which extends the reach of the study to the role of fomites in the transmission of human enveloped viruses like influenza and SARS-CoV-2. The data provided here should be useful in microbial risk assessments to aid in the understanding of different modes of indirect viral transmission.

MATERIALS AND METHODS

Volunteers. Volunteers for this study were enrolled with approval from the Stanford University Research Compliance Office for Human Subjects Research according to IRB-55010. Fifteen volunteers participated per surface, similar to the number of volunteers used in previous studies on virus transfer (11, 19). All volunteers were allowed to participate in the study if they self-reported as healthy, had no visible sores on their hands or fingerpads, and had appropriate building access according to Stanford's COVID-19 Research Recovery Plan. The experiments were conducted in a room isolated from others, a 6-foot distance was maintained whenever possible, and facial masks were worn at all times according to Stanford's COVID-19 Research Recovery Plan. Once volunteers were informed of the risks of the experiment and consented, the age, gender, hand length, and hand breadth of the volunteers were recorded. Hand length and breadth were recorded according to procedures of the National Aeronautics and Space Administration (29). The volunteer group consisted of 20 volunteers, 8 of whom self-identified as cisgender-male and 12 as cisgender-female. Not all of the 20 participants performed experiments with all three surfaces. Instead, 15 volunteers participated per surface. Of the 20 volunteers, 8 performed the experiment with all three surfaces, 9 with two surfaces, and 3 with just one surface.

Virus preparation. Phi6 and MS2 were applied to the surfaces and fingerpads together in the same aliquot to ensure viruses were suspended in an equivalent aqueous matrix. An equivalent aqueous matrix is vital to ensure homogenous transfer conditions between the two viruses so that the effect of virus type can be deduced from the experiments. Each virus was diluted to the preferred titer with TSB (tryptic soy broth; BD Bacto), and then they were mixed in equal proportions. TSB was used as the matrix for the experiments to mimic an organic-rich medium, which better resembles bodily excretions like mucus, saliva, vomitus, and feces than a buffer or water solution.

Phi6 (NBRC 105899) and its host, *Pseudomonas syringae* (ATCC 21781), were obtained from the University of Michigan. To propagate *P. syringae*, 30 ml of nutrient broth (described in the supplemental material) was inoculated with a loopful of *P. syringae* stock from $-80°C$ and incubated with shaking at 75 rpm at $30°C$ for 48 h until experiment use. The propagated host was kept at $30°C$ and used for additional experiments up to 48 h after initial use. Phi6 virus stock was created using the method described in the supplemental material by following Wolfe et al. (19). Briefly, a high-titer plaque assay was run, the soft agar was scraped off, and the virus was eluted into phosphate-buffered saline (PBS; Fisher BioReagents). The virus-PBS mixture was then filtered, concentrated, and stored at $-80°C$.

MS2 (DMS no. 13767) and its host, *Escherichia coli* (DMS no. 5695), were purchased from the DSMZ German Collection of Microorganisms and Cell Cultures. Twenty milliliters of tryptic soy broth (TSB; pH 7.3 ± 0.2) was inoculated with 20 μl *E. coli* stock from $-80°C$ and incubated (without shaking) at $37°C$ until the growth phase was logarithmic (about 6 h), and then it was used immediately for experiments. Typical absorbance when the bacteria reached logarithmic growth was between 0.1 and 0.5 optical density units, as measured with a spectrophotometer at a wavelength of 520 nm. MS2 virus stock was created using the method described in the supplemental material. Briefly, MS2 virus stock preparation follows the same outline described for Phi6.

Surface preparation. Samples of the three surfaces were obtained from Home Depot (East Palo Alto, CA, USA). Stainless steel and plastic were light switch cover plates, while painted wood was poplar cut to approximately the same size as the light switch cover plates and painted with interior acrylic semigloss paint (see Fig. S1 in the supplemental material). Two-centimeter squares were delineated on the surfaces using permanent marker. To sterilize each surface, the surface was washed with antibacterial soap, soaked in a 10% bleach solution, triple rinsed with deionized water, and dried with a Kleenex scientific cleaning wipe (Kimberly-Clark, Irving, TX, USA).

Experimental protocol. (i) Overview. The experimental design of this study was modified from Julian et al. (Fig. 2) (11). The experiment can be broken down into two parts, experiment A and experiment B. The experiments have the same setup but differ in the length of time since last hand wash. Experiment A took place an hour after the volunteer washed their hands with soap and water, while experiment B took place immediately after handwashing. In both experiments, a donor surface, which represents the contaminated surface, was inoculated with the viruses, and the virus inoculum was allowed to dry to mimic the desiccation that can occur during natural contamination events. The donor surface could be one of the three nonporous surfaces tested or could be a fingerpad depending on the direction of transfer. Depending on the volunteer's schedule, with some volunteers an additional second surface was tested immediately after the first. In all instances, the contact event then took place with the recipient surface (the clean surface[s] or fingerpad[s] depending on the direction of transfer). Samples were recovered from both the donor and recipient surfaces. After experiment A, the volunteer washed their hands using the same technique as that in the beginning of the study, and immediately experiment B took place. After experiment B, the volunteer washed their hands a final time and the experiment concluded.

(ii) Detailed experimental protocol. A 2-cm by 2-cm square of donor surface (steel, plastic, wood, or fingerpad) was inoculated with 10 μl of pooled virus stock containing both MS2 and Phi6. Virus stock consisted of TSB with $\sim 10^5$ PFU MS2/ml and between 10^8 PFU Phi6/ml and 10^{10} PFU Phi6/ml (yielding $\sim 10^3$ PFU MS2 and 10^6 to 10^8 PFU Phi6 per 10 μl of pooled virus). The higher Phi6 titer stock was used for fingerpad and painted wood donor surfaces, while the lower Phi6 stock was used for stainless steel and plastic surfaces. The different Phi6 titers were required to obtain countable plaques from the recipient surfaces. Temperature and relative humidity of the room during the experiment were recorded using a ThermoPro TP49 digital hygrometer.

An hour prior to experiment A, volunteers were asked by the technician to wash their hands with antibacterial liquid hand soap (Colgate-Palmolive, New York, NY, USA) for 15 s, rinse them in tap water, and dry them with a Kleenex scientific cleaning wipe (Kimberly-Clark, Irving, TX, USA). We note that 15 s is less than the World Health Organization recommended guideline of 20 to 30 s but is supported by the literature as effective for removal of microbes from hands (30). They were asked to refrain from using the restroom, eating food, and wearing latex gloves until the start of the experiment. For each volunteer, one surface to be tested was chosen through a random number generator from 1 to 3 (1, stainless steel; 2, plastic; and 3, painted wood). An optional second surface to be tested the same day was also randomly chosen from the remaining 2 surfaces. Next, the fingerpad corresponding to each direction of transfer and the fingerpad used as a control were chosen through a random number generator from 1 to 5 (1, thumb; 2, index; 3, middle; 4, ring; 5, pinky). With each volunteer, one fingerpad served as a recipient for the chosen surface (surface-to-fingerpad transfer), one fingerpad served as a donor for the chosen surface (fingerpad-to-surface transfer), one fingerpad served as a recipient for the second optional surface (surface-to-fingerpad transfer), one fingerpad served as a donor for the second optional surface (fingerpad-to-surface transfer), and one fingerpad served as a control (Fig. 2). Collection of control samples, where the virus was not applied to the fingerpad, ensured that there were no viruses present on the hand or surface and no cross-contamination present. The right and left hands served as duplicates

of one another, and as a result the designations were identical for each hand (Fig. 2). The viruses were distributed on the appropriate surface and fingerpads in a grid of small droplets (about 0.75 μl per droplet) for even distribution and were allowed to visibly dry. This grid was adjusted for each fingerpad, as they had unique sizes, but was an approximately 4 by 4 grid for surfaces. For surfaces, the drying time typically took about 30 min, while for fingerpads it took about 5 min.

After the inoculum on the donor surface was visibly dry, the contact event took place. The volunteer contacted the surface for 10 s at a pressure of 25 kPa (0.25 kg/cm^2). The appropriate pressure was administered using a triple-balance beam set to 500 g. This pressure is comparable to a child gripping an object, the pressure of adult fingerpads exerted locally on a hand tool, and studies examining transfer of soil from surfaces to skin (31–33). Upon completion of the contact event, a cotton swab (Fisherbrand) wetted with TSB was used to remove the virus from both the donor and recipient surfaces. The swab was swiped firmly across the surface for 10 s using a sweeping motion. The swab was then placed in 1,000 μl of TSB and vortexed for 10 s.

After experiment A was complete, the volunteer was asked to use alcohol-based hand sanitizer (ABHS) and then wash their hands using the same method they used at the start of the experiments. Immediately after washing, experiment B was initiated using the same surface(s) and the same fingerpad donor/recipient designations as experiment A. Experiment B was carried out in the exact same manner as experiment A. At the end of experiment B, volunteers were asked to use ABHS again and to wash their hands a final time.

After the volunteer concluded the experiment, the samples were vortexed, diluted 1:10 and 1:100 using TSB, and then stored at 4°C for a maximum of 8 h until the plaque assays were performed.

Quantification. To enumerate Phi6 and MS2 in the samples, traditional double agar plaque assays were used. The Phi6 plaque assay followed Wolfe et al. (see the supplemental material) (19). Briefly, soft agar (0.3% agar) was inoculated with 100 μl of sample and 100 μl of *P. syringae* host, and then the mixture was poured onto hard agar plates (2.3% agar). The MS2 plaque assay is based on EPA method 1602 (34). Briefly, soft agar (0.7% agar) was inoculated with 300 μl of sample and 200 μl of *E. coli* host, and then we poured the mixture onto hard agar plates (1.5% agar).

Three dilutions of each sample were assayed, including undiluted, 1:10 dilution, and 1:100 dilution samples. In addition, a negative control for each hand and surface was included for each volunteer. The negative control consisted of performing the contact event with a surface and fingerpad that were not inoculated with the virus, swabbing the recipient surface, and processing the swab sample using the plaque assay described. The viral stock concentration was enumerated in each experiment, confirming the plaque assay was working correctly even if no plaques were observed in the surface transfer results. The Phi6 and MS2 hard agar plates were incubated at 30°C and 37°C, respectively, for 18 h before plaques were counted as PFU. The number of PFU were counted if the number was between 1 and 500. If there were more than 500 PFU, TNTC (too numerous to count) was recorded. If there were no PFU, then a 0 was recorded. In some cases where the highest dilution yielded TNTC, additional dilutions were performed (see the supplemental material). Additional information on the quantification methods as well as justifications for the use of 500 as an upper counting limit are provided in the supplemental material.

Data analysis. The transfer rate was calculated using equation 1. In this equation, the transfer rate (r) is defined as the mean number of PFU times the appropriate dilution factor measured on the recipient surface (R_R) divided by the sum of the mean number of PFU times the appropriate dilution factors recovered from both the recipient surface and donor surface (R_D). Some authors (1, 5, 12–14) use percent transfer to describe transfer rate, which is equal to $100 \times r$%. Dilution factor is defined as 1 for undiluted sample, 0.1 for 1:10 diluted, and 0.01 for 1:100 diluted samples. The recovered number of PFU times the dilution factor was used in the denominator rather than the applied concentration, as desiccation results in a loss of viral titer (11) and we sought to quantify transfer specifically without considering effects of desiccation:

$$r = \frac{R_R}{(R_R + R_D)} \tag{1}$$

A sample is defined as an individually collected swab of the virus. Each contact event results in two samples, one from the fingerpad swab and one from the surface swab. There are two levels of replication when quantifying the samples for each of the 15 volunteers per surface. The first are the biological replicates created by the duplicate hand profiles of each volunteer. The second are the technical replicates created from the multiple dilutions of each sample. For the purpose of the data analysis, no separation of the biological replicates was attempted. All available technical replicates were multiplied by their appropriate dilution factors and averaged to obtain one recovery value from the recipient surface and one recovery value from the donor surface. These are the values used in equation 1. Inclusion of the technical replicates can be approached in many ways other than the one chosen (such as only choosing the dilutions that yielded the lowest transfer rate or only using dilutions between a certain range of PFU numbers). Different approaches were tried in the data analysis, and no differences in results were noted (details not shown).

Data cleaning and the calculation of the transfer rate were performed in MATLAB (R2020a; The MathWorks, Natick, MA, USA). If the PFU count was recorded as TNTC or 0 for either the donor or recipient surface, the data for the transfer event were removed. Descriptive statistics (means, medians, and standard deviations) and statistical modeling functions were calculated in R (35). Beta distributions were fit to the data using a univariate maximum likelihood estimation. The goodness of fit was determined through Kolmogov-Smirnoff tests. An *n*-way ANOVA was used to test the hypotheses that virus type,

surface type, time since last hand wash, and direction of transfer were significant experimental factors of the virus transfer rate. The n-way ANOVA was followed by a Tukey honestly significant difference *post hoc* test. ANOVA assumption testing (including blocking and homoscedasticity) is contained in the supplemental material. A significance level of $\alpha = 0.05$ was used in this assessment.

Data availability. A complete data set is available at https://purl.stanford.edu/xd282zn6829.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.7 MB.

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