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ORIGINAL ARTICLE

Minding the matrix: The importance of inoculum suspensions on finger transfer efficiency of virus

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Abstract

Aims: The aim of this study was to determine how the transfer efficiency of MS-2 coliphage from the toilet seat to hands and fingertip to lip differs according to the suspension of the inoculum.

Methods and Results: Hands were sampled after lifting a toilet seat which was inoculated with MS-2 on the underneath side. MS-2 was suspended in a spectrum of proteinaceous and non-proteinaceous solutions. Transfer efficiencies were greatest with the ASTM tripartite soil load (3.02% ± 4.03) and lowest with phosphate-buffered saline (PBS) (1.10% ± 0.81) for hand-to-toilet seat contacts. Finger-to-lip transfer rates were significantly different ($p < 0.05$) depending on suspension matrix, with PBS yielding the highest transfer (52.53% ± 4.48%) and tryptose soy broth (TSB) the lowest (23.15% ± 24.27%). Quantitative microbial risk assessment was used to estimate the probability of infection from adenovirus and norovirus from finger contact with a toilet seat.

Conclusions: The greatest transfer as well as the largest variation of transfer were measured for finger-to-lip contacts as opposed to toilet seat-to-finger contacts. These factors influence the estimation of the probability of infection from micro-activity, that is, toilet seat adjustment.

Significance and Impact: Viruses may be transferred from various human excreta with differing transfer efficiencies, depending on the protein content.

KEYWORDS

finger transfer, fomites, matrices, risk assessment, virus

INTRODUCTION

Significance of fomite transmission of viral disease

The significance of fomites in the transmission of infectious agents is widely recognized (Boone & Gerba, 2007; Kraay et al., 2018; Stephans et al., 2019). The relative contribution of fomite transmission in comparison to other transmission routes is dependent upon the types of viruses, the degree of viral shedding, human crowding of indoor spaces, and the persistence of viruses on commonly shared surfaces (Boone & Gerba, 2007). While fomite transmission is likely not a major contributor to SARS-CoV-2/COVID-19 transmission (Azimi et al., 2021; Kraay et al., 2021), the right sequence of events and

crowding (Dawson et al., 2021) can result in fomite-transmitted virus-associated disease, such as transfer of virus-containing mucus to an elevator button, subsequent transfer to another person's hand and then to the mouth (Xie et al., 2020).

One public setting that has long been a concern regarding the communal transmission of infectious agents is public restrooms. Restroom sites, specifically toilets, play a major role in the harbouring, persistence and transmission of enteric pathogens to such an extent that even soap and detergents can further spread pathogens within the built environment (Abney et al., 2021). Despite evidence of the contribution of public restrooms to communicable disease burden (Carling et al., 2009), the mechanisms by which contaminated fomites contribute to this public health issue are still poorly understood beyond the aerosolization of toilet water and the deposition of aerosols on restroom floors or surrounding surfaces (Barker & Jones, 2005; Johnson et al., 2013; Sassi et al., 2018). For example, the frequency at which restroom surfaces are touched and the transfer efficiency of microbes, especially considering the unique human excreta matrices such as faeces (diarrhoea vs. solid), urine or blood on surfaces within restroom environments, are relatively unknown. Most microbial transfer efficiency studies to date have used trypticase soy broth (TSB) to simulate an organic load. While this is a matrix that may represent gastrointestinal and respiratory excreta (Anderson & Boehm, 2021; Julian et al., 2010; Lopez et al., 2013; Rusin et al., 2002), this notion has not been validated through comparative assessment and does not represent other matrices of concern in restroom environments, such as urine and faeces. Alternatively, few transfer studies have evaluated the effect of viral suspension matrix on transfer efficiencies of viruses (Dallner et al., 2021; Rheinbaben et al., 2000). There is some preliminary evidence that suspension affects transfer efficiency. Zhao et al. (2019) observed differences in transfer efficiency of *Staphylococcus aureus* and *Escherichia* coli when suspended in water vs. a broth medium, where transfer efficiencies were greater when suspended in broth. Differences have also been measured in viral persistence as a function of the suspension matrix (Fedorenko et al., 2020; Lin et al., 2020; Matson et al., 2020; Pastorino et al., 2020; Sobsey & Meschke, 2003), implying there could be differences in the risks viruses pose as a function of their suspension matrix.

Finger to lip transfer efficiency data - state of the science

Two key moments in considering fomite-mediated risk are (1) transfer efficiency from the fomite-to-finger and (2) Transfer efficiency from the finger-to-mucosal membranes such as lips, eyes, and/or nose. Accurately capturing these events is integral to the advancement of risk modelling for organisms that have a hand-to-facial mucosal membrane route. Rusin et al. (2002) have been the only available finger-to-lip transfer data and the most cited transfer efficiency publication to date (Nicas & Best, 2008; Nicas & Jones, 2009; Wilson, Abney, et al.,2020). These data have also been used to represent hand-to-nose and -eye transfer efficiency, due to lack of nose- and eye-specific transfer efficiencies (Beamer et al., 2015; Canales et al., 2019). The data described in Rusin et al. (2002) however did not include standard deviations (only arithmetic means provided) for the transfer efficiency of microbial indicators that have been used in prior quantitative microbial risk assessment (QMRA) studies (Beamer et al., 2015; Brooks et al., 2012; Cook et al., 2017; Ryan et al., 2014). Others have therefore assumed the standard deviation of finger-to-lip transfer rates to inform a distribution in a risk assessment, using only arithmetic means from Rusin et al. (2002) (Julian et al., 2009).

Study objective

Gathering more finger-to-lip transfer efficiency data in conjunction with understanding differences in transfer efficiency as a function of the suspension matrix will advance the mechanistic understanding of fomite transmission and the evaluation of risk assessments. The goal of this study was to address these gaps by determining the impact of virus suspension matrices on transfer efficiency for two types of contacts: (1) fomite-to-finger, and (2) finger-to-lip.

MATERIAL AND METHODS

Participants

Twelve participants were included in this study. All participants were adults aged 18–75 years and were laboratory personnel protected by the University of Arizona Biosafety Risk Management. Permission was obtained and the protocol was approved by University of Arizona Institutional Research Board prior to the conduct of the study; a human subjects review was not required.

Control wash

Prior to all study activities, the following control handwash was performed. Hands were moistened with 70%

ethanol for 10 s, participants rubbed the alcohol thoroughly over their hands and wrists for 15 s, and then hands were washed with 2 ml of liquid ivory soap (Procter and Gamble) for 30 s, rinsed for 15 s using tap water from a faucet, and dried on paper towels. Prior to inoculation of the upper and lower lip, the area was wiped for approximately 10 s with an alcohol swab/wipe. Neither lip nor finger from each participant were reused in these experimental trials. All samples were collected on the same day for each trial to avoid cross contamination, for finger to lip transfer experiments, negative controls of participants fingers were taken prior to the experiment to ensure there was no cross contamination from the laboratory.

After all study sampling involving the prepared phage inoculum, the following disinfection procedure was performed. Participants' hands were sprayed with 70% alcohol for 10s, the alcohol was rubbed over hand and wrist surfaces for 15 s, and then hands were rinsed under running tap water for 15 s and dried with paper towels. Participants then conducted an Ivory soap-and-water wash for at least 30 s and dried their hands. Participants' upper and lower lips were disinfected by twice wiping the area with an alcohol wipe (10 s each).

Inoculum preparation

Coliphage MS2 (ATCC 15597-B1) and its host *Escherichia coli* (ATCC 15597) were obtained from the American Type Culture Collection (ATCC). These were selected as model microorganisms for pathogenic virus.

MS2 coliphage was prepared as previously described by Rusin et al. (2002) with minor modifications. Briefly, 0.1 ml of phage suspension and 0.5 ml of a log-phase *E. coli* 15597 (host bacterium) culture were added to molten top agar (0.26%) maintained at 55°C. The inoculated top agar (BBL Select Agar) (Becton, Dickinson and Company, Sparks or Difco™) was mixed and poured over the tryptic soy agar (0.40%) (TSA) (Becton, Dickinson and Company, Sparks or Difco™). The solidified agar overlay plates were then inverted and incubated at 37°C for 24 h. Phosphate-buffered saline (PBS)(Millipore Sigma) or tryptose soy broth (TSB)(Becton, Dickinson and Company, Sparks or Difco™) was then added to each plate and maintained at room temperature for 2 h. The PBS or TSB eluent was aspirated and centrifuged (1,000 RPM for 25 min), after which the supernatant was filtered through a 0.22-μm-pore-size Steriflip filter (Millipore Sigma). The coliphage stock (~10^{11}–10^{12} PFU/ml) was titered 24 h before each experiment prior to storage at 4°C.

Fetal bovine serum (FBS) (Corning), bovine serum albumin (BSA) (J.T. Baker, Avantor), mucin (MP Biomedicals) and yeast extract (Alfa Aesar) stock solutions were prepared according to the manufacturer's protocol with PBS and filtered through a 0.22 μm filter prior to use in inoculum preparation. Proteinaceous inoculum matrices of 5% fetal bovine sera and ASTM tripartite soil load were prepared according to the ASTM E1053-20 and E1052-20 protocols (Table 1).

Toilet Seat-to-Finger transfer experiment

Each trial for the four outlined inoculum matrices (PBS, TSB, 5% FBS, ASTM Tripartate mixture) consisted of 8 contacts: fomite-to-finger transfer replicated 6 times, 1 negative control (finger with no inoculum and no touch event), and 1 positive control (tittered inoculum added to finger with no touch event). For each one of these 8 areas around the underneath of the toilet seat, a new 1 cm^2 area of the toilet seat was used to not cross contaminate between areas. The inoculated and swabbed surface area were indicated with 1 cm^2 squares outlined by tape (Figure S1). Per 1 cm^2 sized area, inoculum was spread evenly across the underneath side of the plastic toilet seat (Kohler, Kohler, WI). The concentration of phage added to the fomites was approximately 10^7–10^8 plaque-forming units (PFU)/cm^2 of MS2 in suspensions of PBS (Sigma-Aldrich), TSB (Becton Dickinson and Company), 5% FBS (Corning) or ASTM tripartite soil load standard mixture.

TABLE 1 Composition of viral inoculum matrix for transfer efficiency experiments

Media used to suspend virus added to fomite	Compsition	References
PBS	PBS	Sassi et al. (2015)
TSB	TSB	Rusin et al., (2002); Julian et al. (2010); Lopez et al. (2013); Wilson, Reynolds, et al. (2020)
Tripartite soil-load	PBS, BSA, Mucin, & Yeast Extract Stock	Sattar et al. (2000) ASTM International E1053
5% organic soil load	PBS, 5% fetal bovine serum (FBS)	ASTM International (2020a) ASTM International E1052-20 and ASTM International (2020b) (ASTM E1052-20)

Abbreviations: BSA, bovine serum albumin; PBS, phosphate-buffered saline; TSB, tryptose soy broth.

Details regarding 5% FBS and ASTM tripartite soil load can be found elsewhere (Sattar et al., 2000; ASTM 1053), however, each of these suspensions were made using the PBS MS2 stock where 5% FBS was added or BSA, mucin and yeast extract were added to make the ASTM tripartite soil load mixture. Using a pipet tip, each 10-µl inoculum droplet was placed in the centre and spread over the 1 cm^2 sampling area as the toilet seat was upside down. The positive controls used for each trial were of the same inoculum mixture. The fomites were allowed to dry with the toilet seat remaining in the upside-down position for 30 min. Both the negative and positive controls were sampled after the 8 contact events to accurately capture any cross contamination.

For each of the six fomite-to-finger contact events, a separate finger (index, middle and ring fingers) was used from each hand (i.e. six fingers per participant) so as to not cross contaminate between finger contacts. Six transfers (i.e. fingers) in total for MS2 in PBS, 5% FBS and the ASTM tripartite soil loading for each of the matrices was conducted. 10 transfers were included for TSB suspended MS2 for concerns of incomplete contact, all results were included in the analysis. To assess transfer, the inoculum was placed at the centre of the 1 cm^2 fomite area and spread with the pipette tip. The finger contact was made by placing the right or left-hand finger on the centre of the area underneath the toilet seat, covering the inoculated area of the fomite, and lifting the seat and placing it back down in one motion (~10 s). By lifting the toilet seat from the underneath side up to a 90-degree angle, we anticipated to have relatively equal pressures across trials as the action was gravity dependent.

Toilet seat fomite sampling

Following the contact with the toilet seat, the underneath of the toilet seat in the contact area was sampled using a sterile polyester fibre-tipped applicator swab (Puritan; Hardwood Products Company). Swabs were wetted in 1 ml of PBS (Sigma-Aldrich), and then swabbed using a firm sweeping and rotating motion to ensure that the entire seeded surface area (approximately 1 cm^2) was swabbed. The swab was then placed back into the remaining PBS and vortexed for 5 s.

Finger sampling

Using a polyester fibre-tipped applicator swab (Puritan; Hardwood Products Company) moistened in 1 ml of PBS (Sigma), the index, middle and ring finger pads from each hand of each participant (six finger contact events) were sampled using a sweeping and rotating motion. Subsequently, the swab applicator was placed in the PBS vial and vortexed for 5 s (Lopez et al., 2013).

Finger to lip transfer experiment

On a separate day sampling of each subject's upper and lower lip was performed after 10 s of contact with a fingertip that had been inoculated with each inoculum matrix described above. Positive samples, which were titered inoculum placed on subjects' lips and fingers without a transfer event were collected with the method described above.

The subjects' index and middle fingers were used in this experiment for contact with the upper and lower lip. Subjects' mouths remained open to prevent any cross-contamination. The upper and lower lip of each participant served as two separate surface areas for each trial. A total of 5 µl of inoculum was applied to the assigned finger and allowed to air dry for 30 s. The subject then gently rests their fingertip on the middle of the lower lip for 10 s. The fingertip and area of contact on the lip were sampled using a polyester fibre-tipped applicator swab. The upper lip was sampled with the method described above with the subjects' finger.

Assay enumeration

For phage analysis, the plaque assay overlay technique was used. Three plaque assay replicates were used for each sample within each matrix trial. Dilutions of phage suspension (0.1 ml) followed by log phase host culture (1 ml) were added to molten top agar, the inoculated top agar tubes were mixed and poured over a TSA agar, the solidified agar overlay was inverted, and overlay plates were incubated at 37°C for 24 h. Plated samples were incubated aerobically for 18–24 h at 35 ± 2°C, after which numbers of plaques of MS-2 were enumerated. Numbers reported are the average of the counts from the plates in the range of ≥25 to ≤250 PFU.

Transfer efficiency calculation and statistical analyses

The transfer efficiency (TE) was defined as the number of PFU recovered from recipient surface (in this case, the fingertip for the toilet seat-to-fingertip contact and the lip in the fingertip-to-lip contact) relative to the PFU available for transfer from the donor surface (in this case, the toilet seat for the toilet seat-to-fingertip contact and

TABLE 2 Virus does response

Pathogen	Dose response parameter	Dose response curve	Probability of illness	Study
Norovirus	a = 0.104 b = 32.3	Beta-Poisson	60%	Teunis et. al (2008) (zero aggregation)
Adenovirus 4	K = 6.07E-1	Exponential	50%	Couch et. al (1966a,b,1969) interpreted by Haas et al. (1999)

the fingertip in the fingertip-to-lip contact). The mean counts recovered from the hand/lip areas and fomites/fingertips were determined, and these mean counts were then used to evaluate transfer efficiency. Transfer efficiencies were calculated using Equation 1 (Marples & Towers, 1979; Paulson, 2005; Rusin et al., 2002) and were entered in Microsoft Excel 2020 (Microsoft Corp.). Non-detects were not included in our statistical analyses.

$$TE\,(\%) = \text{PFU recipient surface} \div \text{Total PFU available for transfer} \quad (1)$$

To determine if there were statistically significant ($\alpha = 0.05$) differences in transfer efficiency across suspension matrix type, a nonparametric test, the Kruskal-Wallis rank sum test, was used with a post hoc Dunn's test and Benjamini-Hochberg correction to adjust the *p*-values in order to decrease false significant results given the number of comparisons. The effect size was calculated using the eta squared, based on the H-statistic method were calculated for each analysis, and ranges of magnitude of effect size (i.e. small, medium and large) were informed by M.T. Tomczak and Tomczak (2014). Significant differences ($\alpha = 0.05$) in transfer efficiency between transfer type (toilet seat-to-fingertip and fingertip-to-lip) per matrix were evaluated using the Wilcoxon rank sum test. All statistical analyses were done using R version 4.0.2 (RStudio, 2021).

QMRA of one touch exposure

A Quantitative Microbial Risk Assessment (QMRA) was utilized to observe the effect that transfer efficiency data has on associating risk of infection from Norovirus and Adenovirus using a Monte Carlo simulation of 10,000 iterations.

Single-surface touch

To associate the risk of infection due to each contaminated surface (PFU/ml), a one-touch dose calculation was utilized (Equation 2).

$$\text{Hand Concentration (HC)} = \left[\text{SC} * (Af) * TE\right] \quad (2)$$

Hand concentration (HC) was calculated by multiplying the surface concentration (*SC*) by fraction of the hand used for the contact (Af) touching the fomite and by transfer efficiency (TE), using data from the surface to hand observed in this study and prior literature (Lopez et al., 2013; Rusin et al., 2002) (Equation 2).

Dose

$$\text{One} - \text{surface touch Dose} = \{(HC) * (TE * Af * Ah)\} \quad (3)$$

Dose for a one-surface touch was calculated by the concentration on the hand from touching the fomite (HC) multiplied by transfer efficiency of organisms from the hand to face (or mucosal membrane) and by the fraction of the hand surface area used for the contact and the total area of the hand (Ah) (Lopez et al., 2013; Rusin et al., 2002) (Equation 3).

Dose response

Risk of infection was calculated by inputting doses of one-surface touch and multiple surface touch models into dose–response equations (Table 2). Risk was estimated with respect to one- and three-time exposure events by adjusting the dose based on the assumed frequency of exposure.

Beta-Poisson:

$$\text{P(response)} = 1 - 1\left[1 + (\text{dose}/\beta)\right]^{-\alpha} \quad (3)$$

Exponential:

$$\text{P(response)} = 1 - \exp(-\text{K} \times \text{dose}) \quad (4)$$

RESULTS

Experiment results

Experimental results are summarized in Table 3. For toilet seat-to-finger contacts, average transfer efficiency was the highest for ASTM tripartite soil load and lowest for PBS (ASTM tripartite soil load > TSB > FBS > PBS). For finger-to-lip contacts, PBS yielded the highest transfer

efficiencies, with the lowest being for TBS (PBS > ASTM tripartite soil load > FBS > TSB) (Table 3). Across all suspension matrices, transfer efficiencies differed according to the type of contact, where average transfer efficiencies were greater for finger-to-lip contacts (averages ranging from 23.15% to 52.53%) (Figure 1) than for toilet seat-to-finger contacts (averages ranging from 1.10% to 3.02%) (Figure 2). Using the Wilcoxon rank sum test, significant differences between contact type (fomite-to-hand vs. finger-to-lip) were observed for each MEM: TSB ($p = 0.011$; fingertip-to-lip: mean = 23.15%, toilet seat-to-finger: mean = 2.14), FBS ($p = 0.002$; fingertip-to-lip: mean = 32.85%, toilet seat-to-finger: mean = 1.14), PBS ($p = 0.002$; fingertip-to-lip: mean = 52.53%, toilet seat-to-finger: mean = 1.10%), ASTM tripartite soil load ($p = 0.004$; fingertip-to-lip: mean = 41.00%, toilet seat-to-finger: mean = 3.02%).

Within contact type, fomite-to-hand transfer efficiencies across matrix type were not statistically significantly different (chi-squared = 5.419, df = 3, $p = 0.1436$). However, statistically significant differences in transfer efficiency across matrix types were observed for finger-to-lip transfer efficiencies (chi-squared = 10.687, df = 3, $p = 0.0135$). Specifically, there was a statistically significant difference between PBS and TSB ($p = 0.013$) as well as FBS and PBS (0.048) where PBS transfer efficiency was greater than for TSB and FBS.

There were no transfer efficiencies above 100%, whereas transfer efficiencies >100% have been an experimental challenge in other studies (Lopez et al., 2013). There were 3 out of 144 transfer efficiency replicate plates for the toilet to finger transfer trials that were below the detection limit (LOD) and not included in the analysis, however, there were no plated replicates below the LOD for the finger to lip trials.

TABLE 3 Transfer efficiencies (%) and standard deviation in plaque forming units (PFU)[a]

	Transfer event					
	Toilet seat to finger			Finger to lip		
Inoculum matrix (MS2)	Mean	SD	*n*	Mean	SD	*n*
PBS	1.10	0.81	6[b]	52.53	4.48	6
ASTM tripartite soil load	3.02	4.03	5	41.00	10.98	6
TSB	2.14	1.62	10	23.15	24.27	6
FBS	1.14	1.23	6	32.85	9.83	6

[a]Four inoculum matrices are compared: phosphate-buffered saline (PBS), American Society for Testing and Materials Soil Load Mixture (ASTM tripartite soil load) Tryptose Soy Broth (TSB), Fetal Bovine Serum (FBS). SD and *n* signify standard deviation and number of samples over detection limit.

[b]Some of the sample triplicates were below detection limit but not all.

Risk analysis

The use of transfer efficiencies obtained from the experiments performed in this study resulted in calculated risk of infection for adenovirus and norovirus nearly ten times lower than risk calculated from Lopez et al. (2013) and Rusin et al. (2002) combined (Table 4). Out of the 4 matrices used in this study, ASTM tripartite soil load transfer efficiency resulted in the highest risks of infection for adenovirus (0.049 ± 0.064) and norovirus (0.15 ± 0.22).

DISCUSSION

The suspension matrix was found to effect transfer efficiency (Table 2), where transfer efficiencies for finger-to-lip contacts were statistically significantly different across the different matrices ($p = 0.013$, effsize = 0.384 [large]). Transfer efficiency was greater with an organic load than without. Transfer efficiencies from toilet seat contact were greatest with the tripartite soil load ($3.02\% \pm 4.03$) and lowest with PBS ($1.10\% \pm 0.81$). However, a statistically significant difference between matrices was not observed for toilet seat-to-finger contacts ($p = 0.53$, effsize = 0.35 [small]). This may have been a result of a relatively small sample size ($n = 6$ except for TSB where $n = 10$). Despite lack of statistically significant differences, notable differences in average transfer efficiency were observed (Table 3). Although Dallner et al. (2021) observed that transfer of virus to other surfaces is supported by the presence of proteinaceous solutions, the significance of PBS finger-to-lip transfer in comparison to TSB and 5% FBS MEMs suggest that Dallner et al. (2021) conclusion does not encompass self-inoculation events (finger-to-lip transfer).

To this date, there is a major lack of finger-to-lip transfer studies to the point that Rusin et al. (2002) is the sole reference for self-inoculation transfer efficiency rates to calculate dose for QMRA. Our model used a Monte Carlo simulation approach to account for variation of input variables (i.e. transfer efficiency, hand surface contact, etc.) with 10,000 iterations to associate a 1:10,000 risk level, most commonly used in QMRA and by the U.S. Environmental Protection Agency (U.S. E.P.A.) to determine the need for public health interventions with risk probabilities that exceed this 1 in 10,000 acceptable risk level (0.01%) (Wilson, King, et al., 2020; Rose & Gerba, 1991; Anonymous, 1989). All one-touch models, regardless of MEM transfer efficiency used, exceeded the

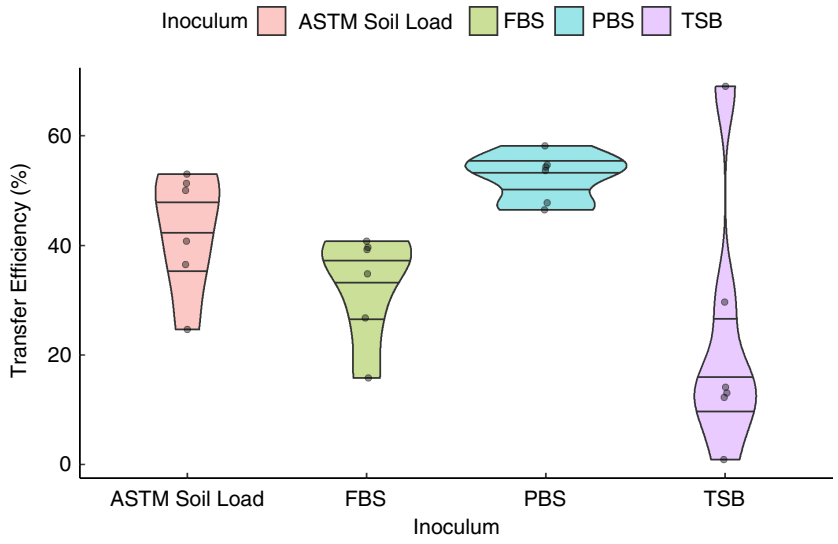

FIGURE 1 Violin plots of finger to lip transfer efficiencies (%) by inoculum matrix with horizontal lines indicating 25th, 50th and 75th percentiles.

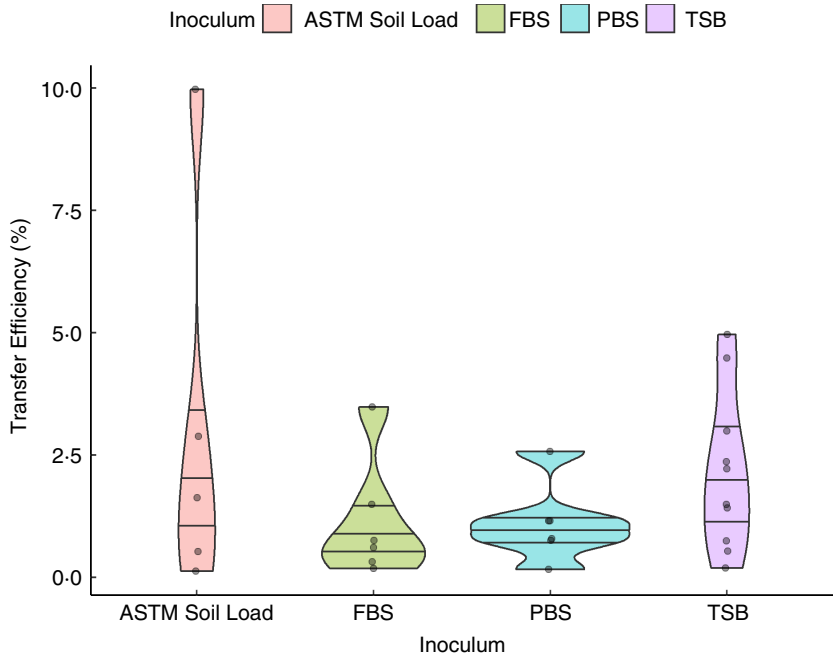

FIGURE 2 Violin plots of toilet seat adjustment transfer efficiencies by inoculum matrix with horizontal lines indicating 25th, 50th and 75th percentile.

acceptable level of risk (0.01%). However, the use of the transfer efficiencies of our experiment resulted in risks close to the risk threshold of 1:10,000 (0.01%) while there was a 10-fold or greater risk of infection probability when risk is calculated with Rusin et al. (2002) for both fomite-to-finger and finger-to-lip transfer efficiency data or with finger-to-lip transfer from Rusin et al. (2002) and fomite-to-finger transfer efficiency from Lopez et al. (2013).

Other limitations in this study include evaluation of effect of surface area with respect to different grip options for adjusting the toilet. Recovery efficiencies evaluated as a function of matrix type was also unexplored in this study but may be insightful in future research.

Regardless of the limitations, these results provide evidence that matrix should be considered in determining transfer efficiency distributions to be used in risk assessments, especially in exposure scenarios where specific matrices are expected (faeces and urine in restroom-specific risk assessments, for example). To our knowledge, this is the first study to investigate the importance of viral inoculum matrix for both finger-to-lip transfer and transfer occurring for specific micro-activities, such as lifting a toilet

TABLE 4 Percent risk of infection for one-touch contact with adjusting the toilet seat with previously reported and current experimental results[a]

Virus	Lopez + Rusin	Rusin	FBS	TSB	ASTM	PBS
Adeno	0.28 ± 0.36	0.50 ± 0.58	0.012 ± 0.018	0.018 ± 0.030	0.049 ± 0.064	0.015 ± 0.021
Noro	0.93 ± 0.10	1.58 ± 1.53	0.045 ± 0.067	0.066 ± 0.11	0.15 ± 0.22	0.056 ± 0.076

Abbreviations: ASTM tripartite soil load, American society for testing and materials soil load mixture; FBS, fetal bovine serum; PBS, phosphate buffered saline; TSB, tryptose soy broth.

[a]Four inoculum matrices are compared.

seat. There is sparse evidence in prior literature suggesting that chosen inoculum matrices are representative of secretions that may contaminate fomites (Rusin et al., 2002; Julian et al., 2010; Lopez et al., 2013; Greene et al., 2015). Even commonly used inoculums such as FBS and components of the ASTM tripartite soil load vary widely by batch (Rossi et al., 1995; Spees et al., 2004). Reported serum albumin relative concentrations in FBS alone are unrepresentative of any reported protein content in bodily fluids (Schenkels et al., 1995; Zheng et al., 2006).

With such inconsistency in selected viral inoculum matrices and suspension solutions, there is a need for standard practices and data-justified selections of matrices to support the creation of "Model Excreta Matrices" (MEM) in transfer efficiency studies. These studies are crucial for improving quantitative microbial risk assessments in their ability to better encompass variability in transfer efficiencies related to suspension matrix. Our controlled laboratory experiment of lifting and lowering the toilet seat with one finger was designed to best inform the modelled behaviour of adjusting the toilet seat within the restroom rather than other more controlled or less-specific activities previously explored which resulted in a nearly ten-fold difference in calculated risk of infection (Lopez et al., 2013; Rusin et al., 2002). By comparing previously used and standardized matrices, we exemplify the effect that different transfer efficiency data has on calculating risk of infection in this study. For example, although TSB has been the most used in prior transfer efficiency studies, there is no literature supporting its claim to be an MEM (Julian et al., 2010; Lopez et al., 2013; Rusin et al., 2002). Human excreta themselves are wholly different from one another concerning the protein composition, with many salivary proteins not being represented in TSB (Reynolds & Chrétien, 1984; Schenkels et al., 1995; Zheng et al., 2006). Differences in nasal secretions alone vary widely in total protein concentrations of same-sex cohorts while longitudinally, individual's mucous volume and protein concentration remain relatively consistent (Rossen et al., 1966). Although viral inflammatory responses lead to increased proteinaceous concentrations by nearly two-fold, baseline variability between individuals has led to the inability to confirm the significant difference of nasal drip between non-infected and infected individuals with rhinovirus (Yuta et al., 1998). Further research is needed to fully understand the components of human excreta most common for enteric pathogen transmission to properly define the best synthetic MEM.

Data are also needed to better understand influences of human behaviour on transfer efficiency (i.e. pressure, contact angle and contact duration), environmental factors (i.e. temperature and relative humidity), and other factors that may influence uncertainty or variability. Standard experimental practices are needed for better use of these experiments. For example, due to the lack of reported standard deviations in Rusin et al., 2002, assumptions about variability in previous finger-to-lip transfer efficiency data have been made (Julian et al., 2009; Kraay et al., 2018).

Evaluation of the survival and transmission of pathogens in various matrices that are representative of human excreta is necessary to fully evaluate the risk of infection and to reduce uncertainty. Previous studies have already assessed the survivability of pathogens in synthetic matrices with inclusion of comparative analyses of multiple matrices, including non-synthetic controls (Abad et al., 1994; Fedorenko et al., 2020; Kasloff et al., 2021; Sizun et al., 2000; Thomas et al., 2014). Although, conclusions have indicated the need for further assessment of more robust MEMs meaning matrices that are supported to closely represent specific human excreta. Currently, the only validated MEM is synthetic faecal matter made to test human waste processing in space—to our knowledge no transfer efficiency study has used this to date (Wignarajah et al., 2006). Transfer efficiencies largely inform quantitative microbial risk assessments from fomite-mediated transmission and accumulation on the hand to a hand to face contact or dosing event (Amoah et al., 2021; Park et al., 2015; Tamimi et al., 2015; Verani et al., 2014; Wilson et al., 2021). MEM are important to explore as human excreta is variable person to person and depending on disease proliferation (i.e. solid vs. diarrhoea). It is important that future research provide justification as to which suspension mixture may serve as a potential MEM for selected exposure event behaviours to better associate pathogen transmission, transfer due to human behaviour, varying types of human excreta, and risk of infection.

This study demonstrated the importance of transfer efficiency experiments to encompass more realistic micro activities, such as adjusting the toilet seat, that are not captured in prior literature to more accurately calculate risk of infection (Lopez et al., 2013; Rusin et al., 2002). To our knowledge, this is one of the first microbial transfer efficiency studies consisting of not only scenario-specific fomite contact types (i.e. lifting the toilet seat) but also containing comparisons of transfer efficiency as a function of virus inoculum matrices of various protein concentrations while examining their impact on associated risk of infection.

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CONFLICT OF INTEREST

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REFERENCES

Abad, F.X., Pinto, R.M. & Bosch, A. (1994) Survival of enteric viruses on environmental fomites. *Applied and Environmental Microbiology*, 60(10), 3704–3710.

Abney, S.E., Bright, K.R., McKinney, J., Ijaz, M.K. & Gerba, C.P. (2021) Toilet hygiene–review and research needs. *Journal of Applied Microbiology*, 131(6), 2705–2714.

Amoah, I.D., Pillay, L., Deepnarian, N., Awolusi, O., Pillay, K., Ramlal, P. et al. (2021) Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities. *International Journal of Hygiene and Environmental Health*, 236, 113807.

Anderson, C.E. & Boehm, A.B. (2021) Transfer rate of enveloped and nonenveloped viruses between Fingerpads and surfaces. *Applied and Environmental Microbiology*, 87(22), e01215–e01221.

Anonymous. (1989) "National drinking water regulations: filtration disinfection; turbidity, giardia, lamblia, viruses, legionella, and heterotrophic bacteria" Final rule, 40 CFR, parts 141 and 142. Federal Registrar 54, 27486–27541.

ASTM International. ASTM E1053-20. Standard practice to assess virucidal activity of chemicals intended for disinfection of inanimate, nonporous environmental surfaces. 2020a; Available at: https://www.astm.org/Standards/E1053.htm. Accessed May 7, 2020.

ASTM International. ASTM E1052-20. Standard practice to assess the activity of microbicides against viruses in suspension. 2020b; Available at: https://www.astm.org/Standards/E1052.htm. Accessed May 7, 2020.

Azimi, P., Keshavarz, Z., Laurent, J.G.C., Stephens, B. & Allen, J.G. (2021) Mechanistic transmission modeling of COVID-19 on the diamond princess cruise ship demonstrates the importance of aerosol transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 118(8), e2015482118.

Barker, J. & Jones, M.V. (2005) The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. *Journal of Applied Microbiology*, 99(2), 339–347.

Beamer, P.I., Plotkin, K.R., Gerba, C.P., Sifuentes, L.Y., Koenig, D.W. & Reynolds, K.A. (2015) Modeling of human viruses on hands and risk of infection in an office workplace using micro-activity data. *Journal of Occupational and Environmental Hygiene*, 12(4), 266–275.

Boone, S.A. & Gerba, C.P. (2007) Significance of fomites in the spread of respiratory and enteric viral disease. *Applied and Environmental Microbiology*, 73(6), 1687–1696.

Brooks, J.P., McLaughlin, M.R., Gerba, C.P. & Pepper, I.L. (2012) Land application of manure and class B biosolids: an occupational and public quantitative microbial risk assessment. *Journal of Environmental Quality*, 41, 2009–2023.

Canales, R.A., Reynolds, K.A., Wilson, A.M., Fankem, S.L., Weir, M.H., Rose, J.B. et al. (2019) Modeling the role of fomites in a norovirus outbreak. *Journal of Occupational and Environmental Hygiene*, 16(1), 16–26.

Carling, P.C., Bruno-Murtha, L.A. & Griffiths, J.K. (2009) Cruise ship environmental hygiene and the risk of norovirus infection outbreaks: an objective assessment of 56 vessels over 3 years. *Clinical Infectious Diseases*, 49(9), 1312–1317.

Cook, E.A.J., deGlanville, W.A., Thomas, L.F., Kariuki, S., deClare Bronsvoort, B.M. & Fèvre, E.M. (2017) Risk factors for leptospirosis seropositivity in slaughterhouse workers in western Kenya. *Occupational and Environmental Medicine*, 74(5), 357–365.

Dallner, M., Harlow, J. & Nasheri, N. (2021) Human coronaviruses do not transfer efficiently between surfaces in the absence of organic materials. *Viruses*, 13(7), 1352.

Dawson, P., Worrell, M.C., Malone, S., Tinker, S.C., Fritz, S., Maricque, B. et al. (2021) Pilot investigation of SARS-CoV-2 secondary transmission in kindergarten through grade 12 schools implementing mitigation strategies—St. Louis County and city of Springfield, Missouri, December 2020. *Morbidity and Mortality Weekly Report*, 70(12), 449.

Fedorenko, A., Grinberg, M., Orevi, T. & Kashtan, N. (2020) Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Scientific Reports*, 10(1), 1–10.

Greene, C., Vadlamudi, G., Eisenberg, M., Foxman, B., Koopman, J. & Xi, C. (2015) Fomite-fingerpad transfer efficiency (pick-up and deposit) of *Acinetobacter baumannii*—with and without a latex glove. *American Journal of Infection Control*, 43(9), 928–934.

Johnson, D., Lynch, R., Marshall, C., Mead, K. & Hirst, D. (2013) Aerosol generation by modern flush toilets. *Aerosol Science and Technology*, 47(9), 1047–1057.

Julian, T.R., Canales, R.A., Leckie, J.O. & Boehm, A.B. (2009) A model of exposure to rotavirus from nondietary ingestion iterated by simulated intermittent contacts. *Risk Analysis*, 29(5), 617–632.

Julian, T.R., Leckie, J.O. & Boehm, A.B. (2010) Virus transfer between finger pads and fomites. *Journal of Applied Microbiology*, 109(6), 1868–1874.

Kasloff, S.B., Leung, A., Strong, J.E., Funk, D. & Cutts, T. (2021) Stability of SARS-CoV-2 on critical personal protective equipment. *Scientific Reports*, 11(1), 1–7.

Kraay, A.N., Hayashi, M.A., Hernandez-Ceron, N., Spicknall, I.H., Eisenberg, M.C., Meza, R. et al. (2018) Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. *BMC Infectious Diseases*, 18(1), 1–13.

Kraay, N.S., Hayashi, M.L., Berendes, D.M., Sobolik, J.S., Leon, J.S. & Lopman, B.A. (2021) Risk for fomite-mediated transmission of SARS-CoV-2 in child daycares, schools, nursing homes and offices. *Emerging Infectious Diseases*, 27(4), 1229–1231.

Lin, K., Schulte, C.R. & Marr, L.C. (2020) Survival of MS2 and Φ6 viruses in droplets as a function of relative humidity, pH, and salt, protein, and surfactant concentrations. *PLoS One*, 15(12), e0243505.

Lopez, G.U., Gerba, C.P., Tamimi, A.H., Kitajima, M., Maxwell, S.L. & Rose, J.B. (2013) Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. *Applied and Environmental Microbiology*, 79(18), 5728–5734.

Marples, R.R. & Towers, A.G. (1979) A laboratory model for the investigation of contact transfer of micro-organisms. *Epidemiology and Infection*, 82(2), 237–248.

Matson, M.J., Yinda, C.K., Seifert, S.N., Bushmaker, T., Fischer, R.J., vanDoremalen, N. et al. (2020) Effect of environmental conditions on SARS-CoV-2 stability in human nasal mucus and sputum. *Emerging Infectious Diseases*, 26(9), 2276–2278.

Nicas, M. & Best, D. (2008) A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. *Journal of Occupational and Environmental Hygiene*, 5(6), 347–352.

Nicas, M. & Jones, R.M. (2009) Relative contributions of four exposure pathways to influenza infection risk. *Risk Analysis*, 29(9), 1292–1303.

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. & Vinjé, J. (2015) Evaluation of a new environmental sampling protocol for detection of human norovirus on inanimate surfaces. *Applied and Environmental Microbiology*, 81, 5987–5992.

Pastorino, B., Touret, F., Gilles, M., deLamballerie, X. & Charrel, R.N. (2020) Prolonged infectivity of SARS-CoV-2 in fomites. *Emerging Infectious Diseases*, 26(9), 2256–2257.

Paulson, D.S. (2005) The transmission of surrogate Norwalk virus—from in animate surfaces to gloved hands: is it a threat? *Food Protection Trends*, 25, 450–454.

Reynolds H.Y., and Chrétien, J. Respiratory tract fluids: analysis of content and contemporary use in understanding lung diseases. *Disease-a-Month*1984;30: 1–103. pmid:6363022https://www.sciencedirect.com/science/article/pii/0011502984900087

Rheinbaben, F.V., Schünemann, S., Gross, T. & Wolff, M.H. (2000) Transmission of viruses via contact in a household setting: experiments using bacteriophage φX174 as a model virus. *The Journal of Hospital Infection*, 46(1), 61–66.

Rose, J.B.& Gerba, C.P. (1991) Use of risk assessment for development of microbial standards. *Water Science and Technology*, 24(2), 29–34.

Rossen, R.D., Schade, A.L., Butler, W.T. & Kasel, J.A. (1966) The proteins in nasal secretion: a longitudinal study of the gammaA-globulin, gammaG-globulin, albumin, siderophilin, and total protein concentrations in nasal washings from adult male volunteers. *Journal of Clinical Investigation*, 45(5), 768–776.

Rossi, S., Bonferoni, M.C., Lippoli, G., Bertoni, M., Ferrari, F., Caramella, C. et al. (1995) Influence of mucin type on polymer-mucin rheological interactions. *Biomaterials*, 16(14), 1073–1079.

RStudio Team (2021). RStudio: integrated development environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/

Rusin, P., Maxwell, S. & Gerba, C. (2002) Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*, 93(4), 585–592.

Ryan, M.O., Haas, C.N., Gurian, P.L., Gerba, C.P., Panzl, B.M. & Rose, J.B. (2014) Application of quantitative microbial risk assessment for selection of microbial reduction targets for hard surface disinfectants. *American Journal of Infection Control*, 42(11), 1165–1172.

Sassi, H.P., Reynolds, K.A., Pepper, I.L. & Gerba, C.P. (2018) Evaluation of hospital-grade disinfectants on viral deposition on surfaces after toilet flushing. *American Journal of Infection Control*, 46(5), 507–511.

Sassi, H.P., Sifuentes, L.Y., Koenig, D.W., Nichols, E., Clark-Greuel, J., Wong, L.F. et al. (2015) Control of the spread of viruses in a long-term care facility using hygiene protocols. *American Journal of Infection Control*, 43(7), 702–706.

Sattar, S.A., Abebe, M., Bueti, A., Jampani, H. & Newman, J. (2000) De-termination of the activity of an alcohol-based hand gel against human adeno-, rhino-, and rotaviruses using the finger pad method. *Infection Control and Hospital Epidemiology*, 21(8), 516–519.

Schenkels, L.C., Veerman, E.C. & Nieuw Amerongen, A.V.(1995) Biochemical composition of human saliva in relation to other mucosal fluids. *Critical Reviews in Oral Biology and Medicine*, 6(2), 161–175.

Sizun, J., Yu, M.W.N. & Talbot, P.J. (2000) Survival of human coronaviruses 229E and OC43 in suspension and after drying onsurfaces: a possible source ofhospital-acquired infections. *Journal of Hospital Infection*, 46(1), 55–60.

Sobsey, M.D. & Meschke, J.S. (2003) Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin. *Technical Report Series—World Health Organization, Geneva*, 70, 19–39.

Spees, J.L., Gregory, C.A., Singh, H., Tucker, H.A., Peister, A., Lynch, P.J. et al. (2004) Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. *Molecular Therapy*, 9(5), 747–756.

Stephans, B., Azimi, P., Toemmes, M.S., Heidarinejad, M., Alen, J.G. & Gilbert, J.A. (2019) Microbial exchange via fomites and implicatins for human health. *Current Pollution Reports*, 5, 198–213.

Tamimi, A.H., Maxwell, S., Edmonds, S.L. & Gerba, C.P. (2015) Impact of the use of an alcohol-based hand sanitizer in the home on reduction in probability of infection by respiratory and enteric viruses. *Epidemiol Infect*, 143, 3335–3341.

Thomas, Y.A., Boquete-Suter, P., Koch, D., Pittet, D. & Kaiser, L. (2014) Survival of influenza virus on human fingers. *Clinical Microbiology and Infection*, 20(1), 58–64.

Tomczak, M. & Tomczak, E. (2014) The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends in Sport Sciences*, 1(21), 19–25.

Verani, M., Bigazzi, R. & Carducci, A. (2014) Viral contamination of aerosol and surfaces through toilet use in healthcare and other settings. *American Journal of Infection Control*, 42, 758–762.

Wignarajah, K., Litwiller, E., Fisher, J.W. & Hogan, J. (2006) Simulated human feces for testing human waste processing technologies in space systems. *SAE Transactions*, 115, 424–430.

Wilson, A.M., Abney, S.E., King, M.F., Weir, M.H., López-García, M., Sexton, J.D. et al. (2020) COVID-19 and use of non-traditional masks: how do various materials compare in reducing the risk of infection for mask wearers? *The Journal of Hospital Infection*, 105(4), 640–642.

Wilson, A.M., King, M.F., López-García, M., Weir, M.H., Sexton, J.D., Canales, R.A. et al. (2020) Evaluating a transfer gradient assumption in a fomite-mediated microbial transmission model using an experimental and Bayesian approach. *Journal of the Royal Society Interface*, 17(167), 20200121.

Wilson, A.M., Reynolds, K.A., Jaykus, L.A., Escudero-Abarca, B. & Gerba, C.P. (2020) Comparison of estimated norovirus infection risk reductions for a single fomite contact scenario with residual and nonresidual hand sanitizers. *American Journal of Infection Control*, 48, 538–544.

Wilson, A.M., Weir, M.H., Bloomfield, S.F., Scott, E.A. & Reynolds, K.A. (2021) Modeling COVID-19 infection risks for a single hand-to-fomite scenario and potential risk reductions offered by surface disinfection. *American Journal of Infection Control*, 49(6), 846–848. doi:10.1016/j.ajic.2020.11.013

Xie, C., Zhao, H., Li, K., Zhang, Z., Lu, X., Peng, H. et al. (2020) The evidence of indirect transmission of SARS-CoV-2 reported in Guangzhou, China. *BMC Public Health*, 20(1), 1–9.

Yuta, A., Doyle, W.J., Gaumond, E., Ali, M., Tamarkin, L., Baraniuk, J.N. et al. (1998) Rhinovirus infection induces mucus hypersecretion. *The American Journal of Physiology-Lung Cellular and Molecular Physiology*, 274(6), L1017–L1023.

Zhao, P., Chan, P.T., Gao, Y., Lai, H.W., Zhang, T. & Li, Y. (2019) Physical factors that affect microbial transfer during surface touch. *Building and Environment*, 158, 28–38.

Zheng, X., Baker, H., Hancock, W.S., Fawaz, F., McCaman, M. & Pungor, E., Jr. (2006) Proteomic analysis for the assessment of different lots of fetal bovine serum as a raw material for cell culture. Part IV. Application of proteomics to the manufacture of biological drugs. *Biotechnology Progress*, 22(5), 1294–1300.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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