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Transfer of bacteriophage MS2 and fluorescein from N95 filtering facepiece respirators to hands: Measuring fomite potential

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ABSTRACT

Contact transmission of pathogens from personal protective equipment is a concern within the healthcare industry. During public health emergency outbreaks, resources become constrained and the reuse of personal protective equipment, such as N95 filtering facepiece respirators, may be needed. This study was designed to characterize the transfer of bacteriophage MS2 and fluorescein between filtering facepiece respirators and the wearer's hands during three simulated use scenarios. Filtering facepiece respirators were contaminated with MS2 and fluorescein in droplets or droplet nuclei. Thirteen test subjects performed filtering facepiece respirator use scenarios including improper doffing, proper doffing and reuse, and improper doffing and reuse. Fluorescein and MS2 contamination transfer were quantified. The average MS2 transfer from filtering facepiece respirators to the subjects' hands ranged from 7.6–15.4% and 2.2–2.7% for droplet and droplet nuclei derived contamination, respectively. Handling filtering facepiece respirators contaminated with droplets resulted in higher levels of MS2 transfer compared to droplet nuclei for all use scenarios ($p = 0.007$). MS2 transfer from droplet contaminated filtering facepiece respirators during improper doffing and reuse was greater than transfer during improper doffing ($p = 0.008$) and proper doffing and reuse ($p = 0.042$). Droplet contamination resulted in higher levels of fluorescein transfer compared to droplet nuclei contaminated filtering facepiece respirators for all use scenarios ($p = 0.009$). Fluorescein transfer was greater for improper doffing and reuse ($p = 0.007$) from droplet contaminated masks compared to droplet nuclei contaminated filtering facepiece respirators and for improper doffing and reuse when compared improper doffing ($p = 0.017$) and proper doffing and reuse ($p = 0.018$) for droplet contaminated filtering facepiece respirators. For droplet nuclei contaminated filtering facepiece respirators, the difference in MS2 and fluorescein transfer did not reach statistical significance when comparing any of the use scenarios. The findings suggest that the results of fluorescein and MS2 transfer were consistent and highly correlated across the conditions of study. The data supports CDC recommendations for using proper doffing techniques and discarding filtering facepiece respirators that are directly contaminated with secretions from a cough or sneeze.

KEYWORDS

Contact; contamination; doffing; FFR; fomite; reuse

Introduction

Personal protective equipment (PPE) use in healthcare settings presents unique challenges in comparison to other industries given the infectious potential of the hazards. PPE can become contaminated when used in the presence of infectious patients or in environments where pathogens are present. Contaminated PPE can serve as a source for pathogens that can be transferred to healthcare workers (HCWs) or the environment through contact.^[1]

For infection control purposes, PPE used in healthcare settings such as gloves, gowns, surgical masks, and

National Institute for Occupational Safety and Health (NIOSH) certified filtering facepiece respirators (FFRs) are generally considered “single-use” devices and are disposed of after each patient encounter.^[2] However, there are situations where the Center for Disease Control and Prevention has recommended that the reuse or extended use of FFRs be considered as one option to conserve supplies.^[3] Extended use of FFRs is the process whereby the wearer dons a FFR and uses it for multiple patient encounters, without removing the FFR in between patient visits. Reuse is the process of repeated

donning and doffing of the same FFR for multiple patient encounters.

The Occupational Safety and Health Administration's (OSHA) regulation for respiratory protection, described in 29 CFR 1910,^[4] lists the requirements to implement a respiratory protection program when engineering controls are not feasible or in conjunction with engineering controls to enhance worker protection. In accordance with 29 CFR 1910, users of tight fitting respiratory protective devices such as N95 FFRs must perform actions that require contact with the filtering surfaces of a FFR. A FFR should be inspected prior to each use to ensure the FFR is suitable for proper function. This requires the user to grasp the body of the FFR while checking the filtering media and tethering straps for signs of damage. Donning the FFR also requires the user to grasp the filtering surface while holding the mask in position on the face while pulling the straps over their head. And, finally, the wearer is required to perform a user seal check to confirm that a satisfactory seal between the face and the FFR is achieved. This is performed by following the FFR manufacturer's instructions, but generally requires the user to place both hands over the filtering surface of the FFR. Contact between ungloved hands and FFRs can occur if the recommended PPE donning and doffing sequences are followed as FFRs are donned before gloves and doffed after removing gloves.^[5] During single use and extended use, the wearer should not come in contact with the contaminated front surface of the FFR if proper doffing procedures, which requires users to remove the FFR by grabbing the tethering straps at the back of the head, are employed.^[5] However, it has been reported that HCWs often improperly doff N95 FFRs by grasping the front of the respirator's potentially contaminated filtering surface.^[6] With FFR reuse, contact with the potentially contaminated surface is necessary for proper use as the wearer must inspect, don and perform a user seal check on a FFR that may have been contaminated during a previous use by the wearer.

This study was designed to characterize the transfer of bacteriophage MS2 and fluorescein, between FFRs and hands during three simulated FFR use scenarios, namely improper doffing (ID), proper doffing and reuse (PDR), and improper doffing and reuse (IDR). FFRs were contaminated with MS2 and fluorescein in either droplets or droplet nuclei. Droplets were used to loosely mimic contamination of a FFR by wet particles via a direct cough or sneeze, while droplet nuclei simulate contamination from dry aerosolized particles that remain airborne for an extended period of time. Droplets are large-wet particles that have been shown to contaminate the outermost surface of the FFR, while droplet nuclei are small-dry particles that penetrate into the filtering medium of the

FFR.^[7] Test subjects mimicked FFR use in healthcare by doffing, inspecting, donning, and performing a user seal check on contaminated FFRs. The study hypotheses are: (1) FFRs contaminated with droplets will lead to greater contact transfer compared to FFRs contaminated by droplet nuclei; (2) contact transfer of MS2 and fluorescein will be greater for FFR reuse scenarios (PDR & ID) compared to ID given the high contact area required to perform a user seal check during reuse; and (3) fluorescein and MS2 contamination transfer will exhibit significant correlation and similar patterns among the statistical tests.

Methods

Test subjects

The study was approved by the NIOSH Institutional Review Board, and all subjects were provided informed consent. A total of 15 subjects were recruited for this study. Subjects were selected between the ages of 18–60. The simulated FFR use procedures were conducted on a head form; therefore, only the subjects' hands were at risk of exposure. Participants were prohibited from participation if they had any open sores, cuts, scrapes, or abrasions on their hands. Participants with a history of skin cancer or sensitivity to UV light were excluded from the study.

N95 filtering facepiece respirator

The N95 3M 1860 model respirator (3M, St. Paul, MN) used for this study is a NIOSH-approved FFR commonly used by healthcare workers for respiratory protection. The 3M 1860 is FDA cleared for use as surgical mask.

Virus, host cells, and plaque assay

Bacteriophage MS2 ATCC 15597-B1 (American Type Culture Collection, Manassas, VA) served as the challenge virus. Bacteriophage MS2 is non-pathogenic to humans and is widely used as a surrogate for gastrointestinal and respiratory viruses for human subject testing. The MS2 was propagated and enumerated using *Escherichia coli* ATCC 15597 (American Type Culture Collection) in ATCC 271 medium (recipe available at www.atcc.org) as the host. Recovery of MS2 virus was quantified through single-layer method plaque assay.^[8] Plates containing visible virus plaques were counted the following day; plate counts falling between 30 and 300 PFU were recorded. Control plates consisting of *E. coli* with MS2 and *E. coli* without MS2 were poured in parallel to the experimental plates to assess the validity of the plaque assay.

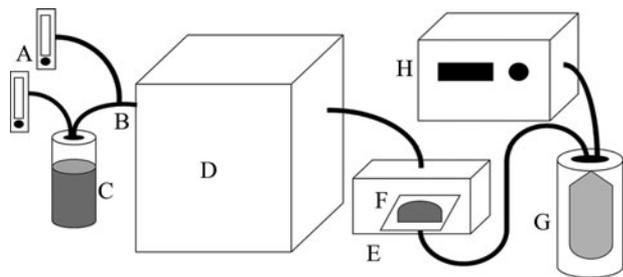


Figure 1. Droplet nuclei contamination system consisting of (A) mass flow meters; (B) aerosol inlet port; (C) collision nebulizer; (D) mixing chamber; (E) loading chamber; (F) filtering facepiece respirator; (G) mechanical lung; and (H) breathing simulator.

FFR contamination with MS2 and fluorescein

Droplet nuclei

A two-chamber system, consisting of an aerosol mixing chamber (Vandiver Enterprises, Zelienople, PA) and a FFR loading chamber (Vandiver Enterprises), was used to apply MS2 aerosols to FFRs (Figure 1). 15 mL of virus suspension, consisting of 10^{10} PFU/mL in 271B medium amended with 0.25% fluorescein (Fisher Science Education, Nazareth, PA) was added to a 6-jet collision nebulizer (BGI, Waltham, MA). A mass flow meter (TSI, Shoreview, MN) upstream from a nebulizer, was used to monitor the air flow of 20 L/min. Dilution air was introduced downstream from the nebulizer at 30 L/min. The nebulizer expelled the virus particles into a 43 L mixing chamber which was connected by hose to the FFR loading chamber. The loading chamber was connected to a mechanical lung (Hans Rudolph, Inc., Shawnee, KS) that was operated by a breathing simulator (Hans Rudolph, Inc., Shawnee, KS) set at 20 breaths per min with a target volume of 1.25 L. A single FFR was sealed between two custom in-house-designed plates which provided an airtight fit to the breathing orifice. The nebulizer was operated for 15 min to allow the system to equilibrate. The breathing simulator was operated for 45 min to contaminate the FFR to a targeted 10^7 PFU/FFR. Each contaminated FFR was sealed in a plastic bag and stored at 4°C overnight. Four FFRs were contaminated for each subject. Three of the FFRs were used to simulate FFR use scenarios and the fourth FFR was used to determine the loading level of virus. Although particle size measurements were not conducted during these experiments, particles with a mass median diameter of 629 nm were produced using the same collision nebulizer, air flow and humidity conditions, mixing chamber, and similar suspension medium in a previous study.^[7]

Droplet

FFRs were contaminated with virus droplets inside a laminar flow biosafety cabinet to loosely mimic wet particles that would be emitted during a direct sneeze or cough.

Each FFR was placed on a head form with the horizontal center seam of the FFR at a height of 26 cm. The droplet spray was generated using a spray bottle, (Fisher Scientific, Pittsburgh, PA) containing 100 mL of MS2 suspension (10^8 PFU/mL and 0.25% fluorescein), a modification to previously described method.^[9] FFRs were sprayed from a distance of 31 cm, with a ring stand and clamp securing the nozzle at a height of 20 cm. FFRs were sprayed five times within approximately 10 sec. The FFRs were placed in a fume hood to dry for 1 hr. Once dry, the FFRs were sealed in plastic bags and stored at 4°C overnight. Four FFRs were contaminated for each subject. Three of the FFRs were used to simulate FFR use scenarios and the fourth FFR was used to determine the loading level of virus. The droplets produced by the spray bottle were not evaluated for particle size and cannot be directly compared to the particle sizes produced by sneezes or coughs. However, an evaluation of the droplet sizes emitted from spray bottles of commercially available cleaning products, similar to that used in this study, produced particles with mass mean diameters above 100 μm .^[10]

FFR loading and deposition profile

FFRs contaminated by both the droplet spray and droplet nuclei systems were assessed to determine the deposition of MS2 among the multiple layers of the FFR. Three circular coupons (18 cm²) were cut out from randomly selected regions of a FFR. Each circular coupon was separated into three layers corresponding to the inner layer (the layer in closest proximity to the wearer when donned), the middle layer (the electret filtering medium), and the outer layer (the layer furthest from the wearer when donned). Each coupon layer was placed in a 50 mL Falcon tube containing 10 mL 1X phosphate buffered saline (PBS) (Fisher Bioreagents, Fair Lawn, NJ) and agitated using a vortexer for 1 min on the highest speed setting. Each FFR coupon layer was removed from the tube and virus recovered from each layer was enumerated by plaque assay. The deposition analysis was conducted in triplicate for both droplet and droplet nuclei-contaminated FFRs.

Virus recovery

FFRs

Virus was recovered from FFRs using a single extraction recovery method. Contaminated FFRs were placed in 500 mL flasks with 200 mL PBS. The flasks were placed in a benchtop shaker and agitated at 300 rpm for 25 min. After the 25 min of agitation, the flasks were removed from the shaker and 20 mL of the virus suspension was transferred from the flasks into 50 mL Falcon tubes (Corning Science, Reynosa, Tamaulipas, MX) for virus enumeration by plaque assay.

To determine the recovery efficiency of the single extraction method, a series of six consecutive virus recoveries were performed on a contaminated FFR. Six 500 mL flasks were each filled with 200 mL of PBS. The FFR was placed into the first flask and agitated in the benchtop shaker using the same parameters as stated above. After shaking for 25 min, the FFR was moved to the second flask and a 20-mL aliquot of the virus suspension was collected from the first flask. The second flask was then placed in the benchtop shaker for 25 min after which the FFR was placed into the third flask and a 20 mL aliquot of the virus suspension was collected from the second flask. This process was repeated on the FFR until all six flasks were used. The six-step recovery determination was performed in triplicate for both droplet and droplet nuclei contaminated FFRs. The single extraction recovery efficiency was estimated as a percentage of the number of viruses recovered from the FFR after the first recovery over the total number of viruses recovered from the six consecutive recoveries.

Hands

Virus was recovered from hands using the glove juice method. The test subjects placed each hand into a nitrile glove (Kimberly Clark, Roswell, GA) and 20 mL of sterile PBS was pipetted into each glove. The gloves were securely sealed by tape at the wrist and each hand was massaged for 60 sec. Each glove was carefully removed from the subject's hand and the PBS containing the recovered virus was pipetted from the glove into a 50 mL Falcon tube. The sample was stored at 4°C for virus quantification.

To determine the virus recovery efficiency of the glove juice method, a known titer of virus was pipetted onto six locations on the hands of the test subjects and recovered using the glove juice method. The finger tips and palm of the hand were contaminated with 5 μ L of virus suspension consisting of MS2 (10^6 PFU/mL) in 271B medium amended with 0.25% fluorescein. The glove juice recovery was conducted using the same methods as described above. Virus recovery efficiency was measured as the percentage of viruses recovered from the hands over the number of viruses placed on the hands.

Fluorescein imaging and quantification

Fluorescein contamination of the hands was quantified using previously described methods.^[11] Images of the subjects' hands were captured under UV-A light using a Nikon DSLR camera (Nikon Corp., Japan) from a fixed distance of 20 cm. Images were imported into Image J software and analyzed using the Lasso tool to delineate the zone of visual and measure the surface area of contamination. The intensity of the fluorescent contamination of

the hand was calculated generating histogram values of the Lasso tool delineated areas and reported as lumens. Each image was analyzed in triplicate.

Simulated FFR doffing and reuse

FFR simulated use scenarios, improper doffing (ID), proper doffing and reuse (PDR), and improper doffing and reuse (IDR), were conducted for both droplet nuclei and droplet spray contaminated FFRs for a total of 6 FFR use scenarios performed by each subject (3 for droplet and 3 for droplet nuclei). The order of the tests was randomized for each subject. Each subject was required to wash their hands for 2 min, as per the World Health Organization hand washing protocol prior to each test.^[12] The subject was instructed on how to perform proper and improper donning/doffing, a user seal check, and FFR inspection. A research technician placed each contaminated mask onto the head form prior to each test. The test subject stood behind the head form, which was mounted on a tripod and adjusted to each subject's height. The subject performed one of three FFR use scenarios as described below and represented in [Figure 2](#). After the subjects performed the FFR use action, images of the

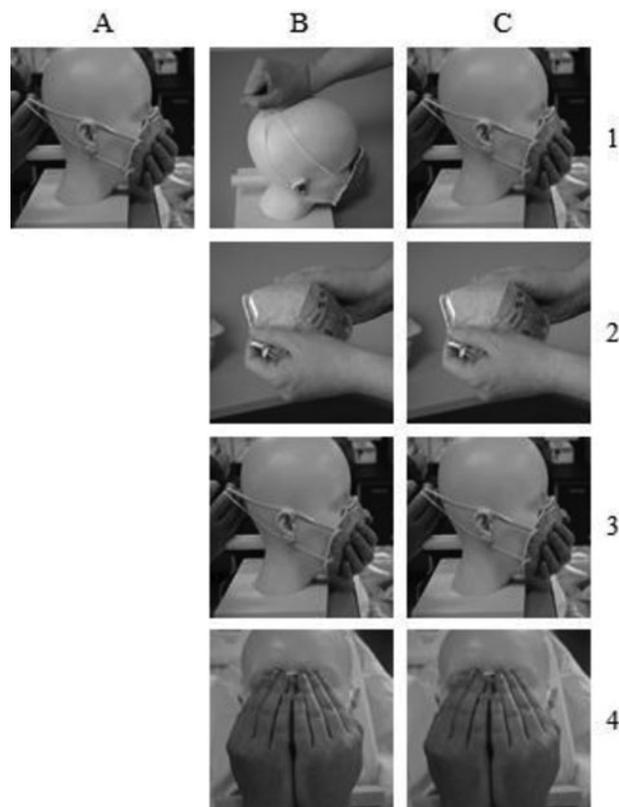


Figure 2. FFR use scenarios. Columns: (A) improper doffing; (B) proper doffing and reuse; and (C) improper doffing and reuse. Contact actions. Rows: 1, doffing, 2, inspecting, 3, donning, and 4, user seal check.

subject's hands were captured and virus was recovered from the hands using the glove juice method.

Improper doffing (ID)

Subjects removed the FFR by grabbing the filtering surface of the front of the mask and placed it in a bin 5 ft away from the head form.

Proper doffing and reuse (PDR)

Subjects removed the FFR from the head form by the grabbing the straps, placed the FFR in a bin placed 5 ft from the head form. The subjects then picked up the FFR from the bin and performed a 5-sec inspection. Following the inspection, the subjects placed the FFR on the head form, and performed a user seal check.

Improper doffing and reuse (IDR)

Subjects removed the FFR by grabbing the filtering surface of the front of the mask and placed it in a bin 5 ft away from the head form. The subjects then picked up the FFR from the bin and performed a 5-sec inspection. Following the inspection, the subjects placed the FFR on the head form, and performed a user seal check.

Data analysis

Percentage transfer (T) of MS2 from FFRs to each hand was calculated by the following equation:

$$T_{(hand)} = \text{PFU}_{(hand)} / \text{PFU}_{(FFR)} \cdot (E_{r(hand)} / E_{r(FFR)}),$$

where $(\text{PFU}_{(hand)})$ is the number of viruses recovered from the hands, $(\text{PFU}_{(FFR)})$ is the number of viruses recovered from the FFR, $E_{r(hand)}$ is the correction factor (1.6) for the recovery efficiency of the glove juice method and $E_{r(FFR)}$ is the correction factor (1.1 and 2.0 for droplet and droplet nuclei contaminated masks, respectively) for the recovery efficiency of the FFRs.

Percentage transfer for FFR use scenarios (ID, PDR, IDR), was determined by summing $T_{(L.hand)}$ and $T_{(R.hand)}$. Statistical significance of MS2 and fluorescein transfer between FFR use scenarios and contamination types was determined by appropriate mean difference tests (Wilcoxon or T-Test).

Results

FFR loading and deposition profile

The average number of viruses deposited on the FFRs was 3×10^7 PFUs and 7×10^7 PFUs for FFRs contaminated using the droplet and droplet nuclei systems, respectively. 99% of the viruses applied to FFRs using the droplet method were deposited on the outer layer of the

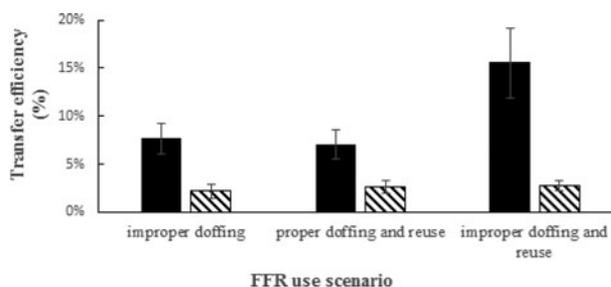


Figure 3. Mean percentage of virus transferred from the FFR contaminated with droplets (solid bars) and droplet nuclei (striped bars) to the hands during improper doffing, proper doffing and reuse and improper doffing and reuse. Error bars represent the standard error of the mean.

FFR, whereas 14% of the viruses deposited on FFRs using the droplet nuclei system were found on the outer layer. Virus was not detected on the inner layer of the FFRs contaminated with droplets or droplet nuclei.

Contamination transfer of FFR use scenarios by contamination type

MS2

For the three types of FFR use scenarios, ID, PDR, and IDR, the average MS2 transfer efficiency measured 7.6, 7.0, and 15.5%, respectively, when handling droplet contaminated FFRs and 2.2, 2.7, and 2.7%, respectively, when handling droplet nuclei contaminated FFRs (Figure 3). To test the statistical significance of the differences in MS2 transfer between droplet and droplet nuclei conditions, Wilcoxon rank sum tests were executed given a violation of the homogeneity of variance assumption. Handling FFRs contaminated with droplets resulted in statistically significant higher levels of virus transfer from FFRs to the subjects' hands compared to droplet nuclei contaminated FFRs for ID ($p = 0.003$), PDR ($p = 0.007$), and IDR ($p < 0.001$).

Fluorescein

For the three types of FFR use scenarios, ID, PDR, and IDR, the average fluorescein transfer intensity measured 6.1, 4.6, and 22.6 lumens, respectively, when handling droplet contaminated FFRs and 3.6, 2.7, and 5.1 lumens, respectively, when handling droplet nuclei contaminated FFRs (Figure 4). Given the violation of the homogeneity of variance assumption the Wilcoxon rank sum test was used to determine if the observed mean differences between droplet and droplet nuclei in the fluorescein condition were statistically significant. Handling FFRs contaminated with droplets resulted in statistically significant higher levels of virus transfer from FFRs to the subjects' hands compared to droplet nuclei contaminated FFRs for IDR ($p = 0.007$). Transfer was not statistically different

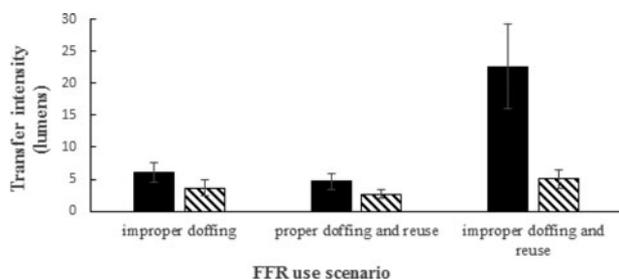


Figure 4. Mean fluorescein transfer from FFRs contaminated with droplets (solid bars) and droplet nuclei (striped bars) to the hands during improper doffing, proper doffing, and reuse and improper doffing and reuse. Error bars represent the standard error of the mean.

between droplet nuclei and droplet derived contamination for PDR and ID.

Contamination transfer of FFR use scenarios within contamination type

MS2

A two-tailed t-test was used in this case given the assumptions were met: normal distribution and equal variances of dependent variable. These assumptions were tested prior to the execution of the test. MS2 transfer for the droplet contaminated FFRs resulting from IDR was statistically greater than transfer during ID ($p = 0.008$) and PDR ($p = 0.042$), while transfer during ID and PDR were not statistically different ($p > 0.05$). For droplet nuclei contaminated FFRs, difference in MS2 transfer did not reach statistical significance when comparing any of the FFR use scenarios.

Fluorescein

T-tests were used given the assumptions for the parametric mean difference approach were met. Similar to the MS2 context, fluorescein transfer for the droplet contaminated FFRs resulting from IDR was statistically greater than transfer during ID ($p = 0.017$) and PDR ($p = 0.018$), while transfer during ID and PDR were not statistically different ($p > 0.05$). For droplet nuclei contaminated FFRs, difference in fluorescein transfer did

not reach statistical significance when comparing any of the FFR use scenarios.

Comparison of statistical outcomes for fluorescein and MS2 transfer data

A Pearson correlation coefficient was derived to examine the level of association between the mean values of MS2 and fluorescein across the experimental conditions of the study. The correlation was very high and significant ($r = .92, p < .001$). This suggests MS2 and fluorescein are nearly equivalent in terms of the rank order of contamination transfer levels across conditions of the study.

Fluorescein and MS2 transfer exhibited similar patterns in the direction of mean-contamination difference among the conditions of the study as well as the results of the statistical tests performed to test the mean difference. Table 1 shows the mean difference test conducted by scenario, statistical method, and indicates whether the direction of the mean difference and the results of the significance tests were consistent between MS2 and fluorescein contamination contexts. Nine of the ten experiments resulted in an equivalent pattern in the direction of mean differences among the conditions of the study. For the single condition that resulted in an inconsistent mean difference direction (i.e., the difference in droplet nuclei contamination levels between the ID and PDR experiments) the mean difference was minimal and consistently non-significant between the MS2 and fluorescein contexts. Eight of ten experiments resulted in an equivalent test of statistical significance. For the two conditions in which there were inconsistent significance values for the mean contamination difference (droplet vs. droplet nuclei in the ID and PDR experiments) the direction of the mean difference was consistent between the MS2 and fluorescein contexts—where the magnitude of the mean difference was greater for MS2 compared to fluorescein.

Parametric analysis of the FFR use scenarios for both droplet and droplet nuclei tests exhibited the same pattern of statistical outcomes for MS2 and fluorescein. The direction of the mean difference between droplet and

Table 1. Statistical significance for each mean difference test for MS2 versus fluorescein data.

Particle type	FFR use scenario	Statistical method	Direction of mean difference	Statistical significance of mean difference
droplet vs droplet nuclei	all scenarios	Wilcoxon rank sum test	consistent	consistent, significant
droplet vs droplet nuclei	ID	Wilcoxon rank sum test	consistent	inconsistent
droplet vs droplet nuclei	PDR	Wilcoxon rank sum test	consistent	inconsistent
droplet vs droplet nuclei	IDR	Wilcoxon rank sum test	consistent	consistent, significant
droplet	ID vs PDR	t-test	consistent	consistent, not significant
droplet	ID vs IDR	t-test	consistent	consistent, significant
droplet	PDR vs IDR	t-test	consistent	consistent, significant
droplet nuclei	ID vs PDR	t-test	inconsistent	consistent, not significant
droplet nuclei	ID vs IDR	t-test	consistent	consistent, not significant
droplet nuclei	PDR vs IDR	t-test	consistent	consistent, not significant

droplet nuclei was similar between MS2 and fluorescein for nine of the 10 scenarios, the results of the nonparametric analysis classified two of the four tests as distinct in terms of the statistical significance of the difference. The two tests for which inconsistencies in statistical significance were found were between droplet and droplet nuclei for the ID scenario and the PDR scenario.

Discussion

This study is one of the first to quantify the transfer efficiency of virus from a NIOSH-approved/FDA cleared N95 surgical FFR to hands of human subjects during donning and doffing exercises that mimic FFR contamination routes and FFR use in healthcare. Recommended PPE donning and doffing sequences used in healthcare require FFRs to be donned before gloves and doffed after removing gloves; thus, it is possible that ungloved hands can come in contact with FFRs.^[5] The virus was applied as droplets and droplet nuclei to simulate potential exposure routes from direct cough or sneeze and air-circulating particles, respectively. The study revealed a few key findings that address, in part, knowledge gaps related to FFR use and reuse in healthcare. First, contrary to our hypotheses improper doffing led to higher transfer levels of contamination transfer compared to the actions associated with reuse scenarios, in particular, performing a user seal check. Second, as hypothesized, droplet contamination exhibited higher transfer efficiencies compared to droplet nuclei contamination for the tested model of FFR. Finally, our results show significant correlation and a similar pattern in results between fluorescein and virus transfer.

Although an elaborate exploration of the physical factors of contact transmission is beyond the scope of this article, a few generalizations about the forces of the FFR use actions can be surmised for the purpose of comparison. Grasping the FFR by the filtering surface while doffing requires a greater force on the FFR compared to the force required to perform a user seal check. However, performing a user seal check requires a greater area of contact than grasping the FFR. Pressure is the quotient of force over area; therefore, grasping the FFR generates more pressure on the contaminated FFR than performing the user seal check. We initially hypothesized that the high surface contact would lead to greater transfer efficiencies; however, the results do not support this initial assumption. This result was not entirely unexpected as Mbithi et al.^[13] examined the influence of contact pressure on contamination transfer and found that a 5-fold increase in pressure led to a 3-fold increase in transfer of hepatitis A virus from metal disks to fingers. Friction, the resisting force between, in this case, the FFR and the hands, may

also lead to higher levels of contamination transfer. Friction forces should be greater for FFR doffing compared to performing a user seal check. In a 2001 study, Sattar et al.^[14] reported that friction increased the level of transfer from fabrics to fingerpads by as much as 5-fold and noted that the effect of friction was not the same across the tested fabric types.

The transfer efficiency of virus from FFRs that were contaminated via the droplet nuclei route were comparable to reported transfer efficiencies for other porous surfaces, while the droplet contaminated masks resulted in transfer efficiencies that are higher than expected for porous materials. Other studies report transfer efficiencies less than 1% for various porous surfaces. Rusin et al.^[15] measured transfer fractions for bacteria (<0.15%) and a bacteriophage (<0.01%) from a variety of porous surfaces, such as laundry, dish cloths, and sponges during task-based activities, such as wringing out the dish cloths and sponges, and transferring laundry. Similarly, Desai et al.^[16] reported transfer efficiencies of less than 1% for a bacterium, *Staphylococcus aureus*, from porous surfaces to pigskin. The method for contaminating the masks may have contributed to transfer efficiencies exceeding 1%.

Contact transfer experiments are often conducted by placing drops of liquid containing a known titer of virus or bacteria onto the donor surface.^[1,17] When using porous donor surfaces, the liquid inoculum penetrates the substrate and the microbial challenge is further dispersed by wicking through the substrate. For studies using FFRs, aerosols are often used to contaminate masks to better mimic the manner that FFRs would be exposed to pathogens while in use. The aerosol is moved via air-flow through the FFR depositing particles on and within the fibrous matrix. Each particle contains multiple viruses which varies depending on numerous factors including particle size and the homogeneity of virus within the aerosol medium. Therefore, each particle that moves from the donor surface to the recipient surface will likely transfer multiple viruses, perhaps leading to increased transfer efficiency. McDonagh et al.^[18] examined transfer efficiency of fluorescein particles deposited as an aerosol on porous and non-porous surfaces to other porous and non-porous surfaces. Porous-donor surfaces were determined to be a significant route of contaminant transfer; however, the effect of the aerosol method of contamination had on the outcome is not evident.

The droplet method of contamination used in this study is similar to the liquid drop methods common to other transfer efficiency investigations; however, the hydrophilic property of the FFR is atypical of more routinely used porous surfaces. The FFR model used in this study is an FDA cleared surgical FFR, which is required

to demonstrate fluid resistance against synthetic blood penetration at various pressures.^[19] The fluid resistance is a result of textile composition or finishes that form a hydrophobic barrier on the surface of the FFR. The hydrophobic property of the outer layer of the FFR causes most of the virus laden fluid to remain on the surface of the mask, whereas most porous surfaces allow the virus or bacteria to become imbedded within the fibers of the substrate, thus limiting contact between the microbes and the recipient surface.

Studies have demonstrated that simulations using fluorescent agents can be useful in visualizing the transfer of contamination from PPE to the user.^[1,20,21] This study sought to examine the efficacy of fluorescein as a possible surrogate for virus in the context of quantifying PPE contamination transfer. The analysis of MS2 and fluorescein contamination demonstrated a similar pattern of findings in terms of the direction of the mean contamination difference between conditions of the study and among the parametric and nonparametric mean difference tests between conditions included in this study. Given that two of the ten statistical tests did not result in equivalent findings of statistical significance between the contamination types, future studies are needed to determine how, when, and where fluorescein contamination serves as an equivalent surrogate to virus contamination. However, it should be noted that the small sample size in the current study may have contributed to the finding of non-significance for the Wilcoxon rank sum test of the difference between droplet and droplet nuclei in the ID and PDR fluorescein conditions. Although subject to future studies, this finding adds to the body of evidence that fluorescein can potentially be used to accurately study PPE contamination and transfer. For example, Tomas et al.^[21] found that the incidence of contamination of hands and forearms during glove removal to be similar for both MS2 and a fluorescent lotion, but did not examine similarity in the challenge level of contamination.

Conclusions

The findings in this article support CDC recommendations that allow for FFR reuse and extended use in a public health emergency. Proper N95 doffing technique is an essential step in limiting self-contamination from contaminated PPE including FFRs, and proved to be effective in this study. Droplet contamination leads to higher contact transfer of contamination; therefore, FFRs contaminated with blood, respiratory or nasal secretions, or other bodily fluids from patients should be discarded.^[22] If N95 FFR reuse is performed with proper technique, the potential for self-contamination can be minimized.

Limitations

For the safety of the study participants, a head form was used to assess contamination transfer from FFR use scenarios. It is possible that using the head form affected the pressure with which the subjects grabbed the FFR for doffing or placed their hands on the surface while performing a user seal check. The contaminating fluid consisting of growth medium, fluorescein, and MS2 was not designed to simulate properties of respiratory secretions, which may affect transfer efficiency. Only one N95 model was used in this study and the interpretation of the results may not be applicable to other FFR models.

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