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Persistence of SARS-Co-V-2 on N95 filtering facepiece respirators: implications for reuse

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Abstract

In response to the shortage of N95 filtering facepiece respirators for healthcare workers during the COVID-19 pandemic, the Centers for Disease Control and Prevention issued guidance for extended use and limited reuse of N95 FFRs to conserve supply. Previously worn N95 filtering facepiece respirators can serve as a source of pathogens, which can be transferred to the wearer while doffing and donning a respirator when practicing reuse. When practicing limited filtering facepiece respirators reuse, to reduce the risk of self-contamination, the Centers for Disease Control and Prevention recommends storing filtering facepiece respirators for five days between uses to allow for the decay of viable pathogens including SARS-CoV-2. This study assesses the persistence of the SARS-CoV-2 strain USA-WA1/2020 on N95 filtering facepiece respirators under controlled storage conditions for up to 5 days to inform the Centers for Disease Control and Prevention guidance. Coupons excised from six N95 filtering facepiece respirator models and glass slide coverslips were inoculated with the virus in a defined culture medium and in human saliva and stored at 20 °C and 20%, 45%, and 75% relative humidity. Statistically significant differences in SARS-CoV-2 half-lives were measured among the tested humidity levels with half-lives decreasing from an average of approximately 30 hr at 20% relative humidity to approximately 2 hr at 75% relative humidity. Significant differences in virus half-lives were also observed between the culture medium and saliva suspension media at 20% and 45% relative humidity with half lives up to 2.9 times greater when the virus was suspended in cell culture medium. The 5-day storage strategy, assessed in this study, resulted in a minimum of 93.4% reduction in viable virus for the most challenging condition (20% relative humidity, cell culture medium) and exceeding 99% reduction in virus at all other conditions.

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Keywords

Contamination; COVID-19; respiratory protection

Introduction

The Centers for Disease Control and Prevention (CDC) confirmed the first COVID-19 case in the United States on January 20, 2020, and by mid-March personal protective equipment (PPE) shortages were being experienced across the United States (Jacobs et al. 2020). PPE plays a vital role in the hierarchy of protection for healthcare workers and the lack of PPE has been identified as a risk factor for healthcare worker SARS-CoV-2 infection (Mhango et al. 2020). CDC NCIRD, DVD (2020a) recommends the use of NIOSH-approved N95 or more protective respirators for healthcare workers caring for patients infected with SARS-CoV-2. The most-used respirator in healthcare is the N95 filtering facepiece respirator (FFR) (CDC NCIRD, DVD 2020b). FFRs are single-use respirators that should be discarded after each patient encounter under normal circumstances. This practice can create a high demand for N95 FFRs particularly during large outbreaks of respiratory infectious diseases. Coupled with supply shortages created by a lack of melt-blown textiles that are used in the construction of many FFRs (Kates 2020) and complex supply structures (Gereffi 2020), the increased demand for FFRs during the COVID-19 pandemic has precipitated the need for optimization strategies to maintain N95 FFR supply. Optimization strategies published by CDC NCIRD, DVD (2020b) offer a continuum of options based on FFR supply status, e.g., when supplies are stressed, running low, or exhausted. One strategy that is recommended by CDC NCIRD, DVD (2020c) when there is a known shortage of FFRs is limited FFR reuse, the practice of using the same N95 FFR for multiple encounters with patients but removing it after each encounter. Limited FFR reuse may also include FFR decontamination, a process to inactivate or reduce the number of pathogens entrapped on the FFR using chemical or physical methods (CDC NCIRD, DVD 2020c).

As an alternative to chemical or physical decontamination of FFRs, CDC NCIRD, DVD (2020c) recommends a passive decontamination strategy which requires that a used N95 FFR be placed in a breathable container such as a brown paper bag for a minimum of five days to allow for virus decay in the ambient environment. This recommendation was based on a study that showed that SARS-CoV-2 could maintain viability for 72 hr on steel and plastic surfaces (van Doremalen et al. 2020). Following the publication of the CDC recommendation, others have conducted investigations into the persistence of SARS-CoV-2 on a variety of surfaces including FFRs and facemasks (Chin et al. 2020, Kasloff et al. 2021). Chin et al. (2020) assessed the viability of SARS-CoV-2 on several surfaces including surgical masks. SARS-CoV-2 in cell transport medium was deposited as droplets onto the surfaces of surgical masks and viability was assessed over a period of up to 7 days at 22 °C and 65% relative humidity (RH). Viable virus was detected on the outer surface of the surgical mask on day seven. Kasloff et al. (2021) evaluated the persistence of SARS-CoV-2 on a variety of PPE including N95 and N100 FFRs at 20 °C and 40% RH. SARS-CoV-2 suspended in a medium designed to simulate virus-containing particles shed by infected individuals (Sattar et al. 2003) was recovered from the FFRs for up to 21 days.

These studies were limited by the number of FFR models, the type of inoculation media, and the temperature and humidity conditions evaluated. Moreover, these investigations into the persistence of SARS-CoV-2 on PPE, including FFRs, used high titers of virus challenges that may not be representative of contamination loads experienced in healthcare settings.

This study assessed the persistence of SARS-CoV-2 suspended in human saliva or cell culture medium at three RH conditions, 20, 45, and 75% RH, on four models of NIOSH-approved and FDA-cleared surgical N95 FFRs and two models of NIOSH-approved N95 FFRs. The study's hypothesis is that SARS-CoV-2 will persist for longer periods in nutrient-rich cell culture medium and under low RH conditions as demonstrated for other coronavirus strains (Chan et al. 2011, van Doremalen et al. 2013). The relevance of the findings of this laboratory study and similar laboratory investigations to the clinical environment, considering the effects of virus suspension medium, RH, surface type, and level of FFR contamination, were assessed to inform FFR use strategies during public health emergencies such as the COVID-19 pandemic.

Methods

Virus propagation

The virus used in this study is the Severe Acute Respiratory Syndrome (SARS)-Coronavirus CoV-2 strain USA-WA1/2020 (WA1), obtained from BEI Resources (Manassas, VA). The virus master stock was harvested in complete cell culture medium consisting of Dulbecco's Minimum Essential Medium (DMEM; Sigma Cat. No. 51416 C, St. Louis, MO) amended with penicillin-streptomycin (Sigma Cat. No. P4333) and 5% fetal bovine serum (Sigma Cat. No. F4135). Stocks were produced in the Vero E6 cell line (African green monkey kidney cells; ATCC CRL-1586, Manassas, VA) using cell monolayers grown in T-flasks (Sigma Cat. No. CLS3291; or equivalent) at 37 °C with 5% carbon dioxide. Cell lysate supernatant was harvested after approximately 72 hr incubation or when cytopathic effects (CPE) were observed on the Vero E6 cells. Resulting SARS-CoV-2 working stocks had titers of approximately 10^6 tissue-culture infectious doses [$TCID_{50}$] per milliliter, and the material was stored as single-use vials at ≤ −80 °C.

Test coupons

A total of six NIOSH-approved FFR models were evaluated for virus persistence in this study: (1) four NIOSH-approved and FDA-cleared surgical N95 FFRs (3M 1860, 3 M VFlex 1804, Moldex 1512, and Moldex 2200 G) and (2) two NIOSH-approved N95 FFRs (3M 8210 and 3M 8511). In addition, glass coupons (microscope cover slides; Fisher Cat. No. 12-545 F, Waltham, MA) were included as a test substrate as a non-porous control. The FFR samples were prepared by excising rectangular coupons (2 × 5 cm) from unused FFRs.

Coupon contamination and storage conditions

The virus was suspended in either complete cell culture medium (described above) or saliva (human saliva, pooled gender; BioIVT, Hicksville, NY) and applied to the 2 × 5 cm FFR and glass slide cover coupons in ten droplets (10 μL each droplet) under ambient conditions (20–22 °C and 30–50% RH). The total deposition of SARS-CoV-2 was approximately 1×10^5

$TCID_{50}$ across the outer surface of the FFR coupon. This contamination load was selected to demonstrate a minimum of 3 log_{10} reduction in virus. Following inoculation, coupons were allowed to dry under ambient conditions to mitigate the potential for loss of the droplet inoculum during transfer to the brown paper bags. After the coupon was placed in the bag, the bag was closed and then stored at 20–22 °C and at 20% (15–25%), 45% (30%–50%), and 75% (70–75%) RH. The ambient relative humidity of the laboratory was 45% (30–50%) and was maintained by central HVAC control. Relative humidity of 20% was achieved with the use of a desiccant (Drierite, 8 mesh; W. A. Hammond Cat. No. 23005, Xenia, OH) in combination with a fan. A saturated salt solution (NaCl Sigma Cat. No. S76533) was used to raise relative humidity within a chamber to 75%. Relative humidity and temperature were measured and documented using a HOBO MX1101 data logger (Onset, Bourne, MA).

Virus on the FFR coupons and glass slides were extracted after 0, 1, 24, 48, 96, and 120-hr timepoints. Additional assessments were performed after 4, 6, and 12 hr post-drying when more data points were needed to calculate the virus half-life under conditions that resulted in shortened persistence. Virus persistence was evaluated in triplicate for each tested surface, timepoint, and condition. Some conditions were not tested for all FFR models due to limited supply of FFRs available for research purposes.

RH and temperature were measured and documented using a sensor Onset (Bourne, MA) Model No. HOBO, Part No. MX1101. The temperature (20–22 °C) was the ambient level in the laboratory

Virus extraction and analysis

Following exposure, coupons were removed from the paper bags and placed into individual 50mL conical tubes containing 10mL extraction buffer (DMEM+penicillin-streptomycin + 2 to 5% fetal bovine serum). The conical tubes were agitated on a platform shaker at 200RPM for 15 min, and the extracts transferred to a concentrator (Spin-X UF Concentrator, Corning Cat. No. CLS431491) and centrifuged at 4,000 × g for 10 min in a swinging bucket rotor until the 10mL starting volume was concentrated to approximately 0.5 mL. Media was added to equilibrate all washed retentates to approximately 2mL. Virus viability was assessed by TCID50 assay in Vero E6 cells by inoculating samples in quintuplicate onto a single 96-well plate at ≥70% cell monolayer confluency. Plates were incubated at 37 ± 2 °C and 5 ± 2% carbon dioxide for 72 ± 4 hr, then observed microscopically for CPE. Observations for CPE were used to quantitatively calculate the viral titer for each sample.

Extraction efficiency was assessed for two representative FFR types, a NIOSH-approved and FDA-cleared surgical N95 FFR (3M 1860) and a NIOSH-approved N95 FFR (3M 8511), and the glass control surface relative to a direct spiking and quantification of the extraction medium. While the mean virus value for the extraction medium was 6.2 log_{10}, the 3M 1860, the 3M 8511, and the glass control surface mean log virus values were 6.0, 6.1, and 6.0 log_{10}, respectively.

Statistical analysis

R and RStudio with the tidyverse (Wickham et al. 2019) and broom (Robinson and Hayes 2018) packages were used to perform regression of the natural log of the fractional

recoveries of virus as functions of exposure duration in hours. The data points for all replicates were used individually and were not averaged. Least-squares methods were used to fit the models and determine the rate of reduction, k; the absolute value of k was divided by 0.693 to calculate the half-life of the variables. The r^2 value was used to assess goodness of fit of the models.

A two-way analysis of variance (ANOVA) model was fit to the log-transformed (base-10) half-life with main effects for RH and test matrix and their interaction. Tukey's multiple comparisons procedure was used to compare group means using SAS Software Version 9.4 for Windows (SAS Institute, Cary, NC).

Results

The calculated viral half-lives along with viral decay curves are shown in Figure 1. Higher humidity resulted in significantly shorter ($p<0.05$) half-lives regardless of suspension medium or N95 model. Comparisons of geometric mean (GM) half-life between suspension media at each RH level show that the half-life for SARS-CoV-2 in culture medium was 2.3 to 2.9 times greater than in saliva at 20% and 45% RH, but not significantly different at 75% RH, where half-life values were short regardless of suspension medium (Table 1).

The shortest half-life observed across all conditions was 1 hr for virus suspended in saliva, inoculated on the 3M 8511 mask and stored at 75% RH; the longest half-life was 64 hr for virus suspended in culture medium, inoculated on the Moldex 2200 mask and stored at 20% RH. At 75% humidity, SARS-CoV-2 virus suspended in either cell culture medium or saliva remained viable and detectable less than 24 hr before falling below the assay limit of quantification (13 $TCID_{50}$/mL), achieving a two- to three-log_{10} reduction in virus viability.

Discussion

This study assessed the effect of suspension medium (cell culture medium, human saliva) and RH (20%, 45%, and 75%) on the persistence of SARS-CoV-2. The results of this study support the hypotheses that SARS-CoV-2 persists for longer periods of time in (1) nutrient-rich cell culture medium compared to human saliva and (2) in low humidity similar to other coronavirus strains (Casanova et al. 2010, Chan et al. 2011). Assessing the relevance of the findings of this laboratory study and similar laboratory investigations to the clinical environment is important for the development and improvement of FFR use strategies during public health emergencies.

Effect of suspension medium

The effect of suspension medium on viral persistence has been shown previously (Firquet et al. 2015) and a recent study by Pastorino et al. (2020) shows that protein and other nutrient constituents of the cell culture medium prolong the persistence of SARS-CoV-2 on surfaces. The results show that the nutrient-rich medium was more favorable to SARS-CoV-2 viability compared to saliva, as evident in the 20% and 45% RH trials. Others have used nutrient-rich media to test the persistence of SARS-CoV-2 on surfaces including surgical masks and N95 and N100 FFRs. Chin et al. (2020) recovered viable SARS-CoV-2 from the exterior

of surgical masks after 7 days of storage at 22 °C and 60% RH. The half-life of the virus, applied in 5 μL droplets of culture medium, was approximately 16 hr which is similar to the half-life reported in this study for the 45% RH tests conducted using cell culture medium and approximately two times the half-life of SARS-CoV-2 in human saliva. Transport media, although undefined by Chin et al., is a nutrient solution used to maintain the viability of microbiological specimens and is likely similar to the cell culture medium used in this study. Kasloff et al. (2021) measured persistence of SARS-CoV-2 on a N95 and a N100 FFR when stored in 20 °C with 35–40% RH. The log reductions reported in Kasloff et al. (2021) equate to a half-life of approximately 30 hr for each FFR type. This is similar to the reported half-life of SARS-CoV-2 in cell culture medium and about 2.5 times the half-life reported for SARS-CoV-2 in human saliva at 20% RH for this study.

The applicability of culture media and human saliva as surrogates for the droplets released from individuals infected with SARS-CoV-2 is not clear. Saliva is typically 99.5% water with inorganic and organic constituents comprising about 0.5% (w/v) (de Almeida et al. 2008). Sputum is highly variable and is comprised of roughly 90–95% water, 4–9% (w/v) organic constituents, and 1% (w/v) electrolytes (Bansil and Turner 2018). The ratio of water, organic, and inorganic constituents of the cell culture medium used in this study is 94%, 5.8%, and 0.2% (w/v), more like sputum than saliva; however, the specific organic and inorganic constituents vary. Moreover, both saliva and sputum contain antibodies and other proteins that are part of the body's response to infection. Matson et al. (2020) measured the viability of SARS-CoV-2, suspended in nasal mucus and in sputum, placed onto polypropylene disks. At 21 °C and 40% RH, the mean half-life of SARS-CoV-2 was reported to be 3.1 hr. Under similar conditions, this study reports a half-life of roughly 16 hr on the surfaces of FFRs which are also made of polypropylene. The difference in virus viability reported on similar substrates under similar temperature and humidity conditions suggests that cell culture medium may not be representative of the constituents typical of respiratory secretions and emphasizes the importance of selecting the proper suspension medium to assess persistence.

Effect of humidity

The effect of RH on SARS-CoV-2 persistence in this study is clear, with virus persistence greatly reduced at higher RH. This trend has been confirmed by other investigations into SARS-CoV-2 persistence (Biryukov et al. 2020) and for other coronaviruses including SARS-CoV (Chan et al. 2011) and mammalian coronaviruses (Casanova et al. 2010). Therefore, storage conditions for reused N95 FFRs in healthcare facilities will influence the persistence of pathogens including SARS-CoV-2.

The National Fire Protection Association (NFPA) standard 99-2012 provides guidance for temperature and humidity control for specific spaces in healthcare facilities and all new construction must comply with the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) standard ASHRAE 170-2008 as referenced in NFPA 99-2012. Hospital rooms typically have a RH requirement between 20% and 60%, although most rooms only have a maximum limit or no requirement for RH (American National Standards Institute, American Society of Heating 2013). Patient care rooms and airborne

infection isolation room a maximum limit of 60% RH with no lower limit. Functional spaces such as decontamination, laundry, soiled linen sorting, and storage, and janitor's rooms do not have a requirement for RH (American Society of Heating 2013). There is limited data about where hospitals are storing used FFRs, but a review of Nebraska Medicine's N95 decontamination procedure shows used FFRs are stored in a soiled utility room which would not have a RH requirement as per standard ASHRAE 170-2008 (Lowe et al. 2020).

Within the 20–60% RH range requirement for healthcare facilities, this study evaluated persistence at 20% and 45% RH. The mean and range of half-lives for SARS-CoV-2 at 20% RH were 37.6hr (22–64 hr) and 12.4 hr (9.4–16 hr) when suspended in cell culture and saliva, respectively. At 45% RH, the mean halflives measured 15.6 hr (13–18 hr) and 6.9 hr (4.2–11 hr), when suspended in cell culture and human saliva, respectively.

This study's results are comparable to half-lives of SARS-CoV-2 estimated using the Department of Homeland Security's SARS-CoV-2 decay rate calculator for surfaces, a tool designed to inform risk assessment conducted by occupational safety and health professionals (https://www.dhs.gov/science-and-technology/sars-calculator). Setting the temperature at 23.3 °C, the lowest temperature available on the calculator, the half-lives reported for 20% and 45% are 14.9 and 11.1 hr, respectively. The calculator derived half-lives are shorter than reported in this study for the cell culture medium trials but longer than the measured half-lives in saliva. The calculated half-lives demonstrate an inverse relationship between virus persistence and humidity which is consistent with the findings of this study. A half-life of 15 hr would result in roughly 99.6% reduction, or greater than 2.3 $\log_{10}$ reduction in virus after 5 days of storage.

Effect of surface

Studies have shown that viruses, including SARS-CoV and SARS-CoV-2, persist for longer periods of times on nonporous surfaces compared to porous surfaces (Vasickova et al. 2010, Aboubakr et al. 2020). However, persistence studies often compare virus stability on porous and nonporous surfaces that are constructed of dissimilar materials, which precludes a direct comparison of the effects of surface porosity (Bean et al. 1982, Tiwari et al. 2006, Whitworth et al. 2020). In this study, the half-lives of SARS-CoV-2 on glass slide slipcovers comparable of the half-lives measured on the FFRs for each tested humidity. This suggests that virus persistence of FFRs may not align with the generalization that virus persistence is shorter on porous materials. FFRs are largely comprised of polypropylene filtering materials (Fisher and Shaffer 2014). van Doremalen et al. (2020) reported a half-life of roughly 16 hr for SARS-CoV-2 deposited in an unspecified suspension on non-porous polypropylene at 40% RH, which is similar to the results on the porous polypropylene FFRs stored in 45% RH reported for this study. These results suggest that material type may be more important than porosity. Kasloff et al. (2021) reported similar stability profiles for SARS-CoV-2 deposited on an N95 and an N100 FFR. The limited research conducted on SARS-CoV-2 persistence on surfaces, including porous and non-porous polypropylene, suggests that the decay profile of the virus will be similar for all FFRs constructed of polypropylene materials.

FFR contamination level

The persistence of viruses such as SARS-CoV-2 is determined, in part, by the given half-life of the virus for specific environmental conditions. As viruses demonstrate a rate of decay, higher initial starting titers would result in longer persistence. For laboratory assessments, high starting titers are used to provide the resolution required to calculate the half-life of the virus but may not be representative of the viral contamination on FFRs used in clinical settings. Ong et al. 2020 reported no detectable SARS-CoV-2 on a small sample of N95 FFRs used during patient care. Similar investigations on FFRs and facemasks, worn for clinical care during influenza season, found minimal or no contamination (Ahrenholz et al. 2018, Rule et al. 2018). Limited studies have reported low levels of aerosolized SARS-CoV-2 in clinical settings (Liu et al. 2020) and the aerosol concentration of influenza virus has been shown to correlate with mask contamination in laboratory settings (Fisher et al. 2014). Moreover, the Occupational Safety and Health Administration (OSHA) assigned protection factor for a properly fit tested N95 FFR used in an OSHA specified respiratory protection program is 10, which is a 10-fold reduction in exposure. High levels of mask contamination may indicate the need for higher levels of respiratory protection.

There are several limitations of this research including the limited number of NIOSH-approved filtering facepiece respirator models and manufacturers evaluated in this study. Each respirator has its individual set of construction materials and characteristics that could impact the SARS-CoV-2 persistence. Another limitation is that the persistence of SARS-CoV-2 on FFR components such as straps, headbands, and nose bridge materials was not evaluated in this study. This study did not investigate the respiratory protection performance of FFRs including fit and filtration. Even in the context of these limitations, these results suggest that the current CDC recommendations for storing FFRs contaminated with SARS-CoV-2 in a paper bag for 5 days prior to reusing will reduce the risk of contact exposure.

Conclusions

The results of this study show that SARS-CoV-2 persistence is shortened under high RH. A minimum of 93.4% reduction in SARS-CoV-2 was measured for all tested humidity conditions, including the minimum permissible RH for any type of room within a healthcare facility as per ASHRAE standards. Storing reused FFRs in areas of the hospital that have higher temperature and RH can increase the efficacy of the storage decontamination method.

The reports of prolonged viability of SARS-CoV-2 on surfaces, including PPE, described in laboratory investigations, may not be relevant to the clinical environment given persistence is influenced by the starting viral titer. The high titers of the SARS-CoV-2 inoculum, required for persistence studies, have resulted in the viable SARS-CoV-2 detection on tested PPE over weeks. The likelihood that clinically used FFRs are contaminated with the titers of virus used in persistence studies is low given the measured and reported contamination levels of clinically used FFRs in the literature. Higher levels of contamination may indicate the need for higher levels of respiratory protection. Lower contamination levels would lead to shorter periods of persistence. Moreover, the use of cell culture medium to inoculate

FFR surfaces provides a favorable environment for the virus and is not representative of respiratory secretions that may envelope the virus in clinical settings.

This research and the results reported in other investigations of coronavirus persistence, suggest that it is prudent to store reused FFRs in areas of the hospital where temperature and RH conditions are higher than the 22 °C and 20% RH conditions evaluated in this study or in rooms where temperature and RH can be adjusted. It should be noted that prolonged storage in high relative humidity conditions may increase the potential for fungal growth or compromise the performance of the FFR. These findings are consistent with the current CDC recommendations to store used FFRs in a paper bag for five days prior to reusing to reduce contamination and the risk of contact transfer while handling reused FFRs, but it is important to follow proper doffing and handling procedures and perform hand hygiene to further minimize contact transmission.

Future studies should assess the persistence of SARS-CoV-2 deposited onto FFRs as aerosols and suspended in other respiratory secretions such as sputum. An investigation of SARS-CoV-2 viability on FFRs exposed to simulated exhaled breath is prudent given the effects of temperature and humidity on viral persistence. These findings may have important infection prevention and control implications for workers conducting SARS-CoV-2 diagnostic testing.

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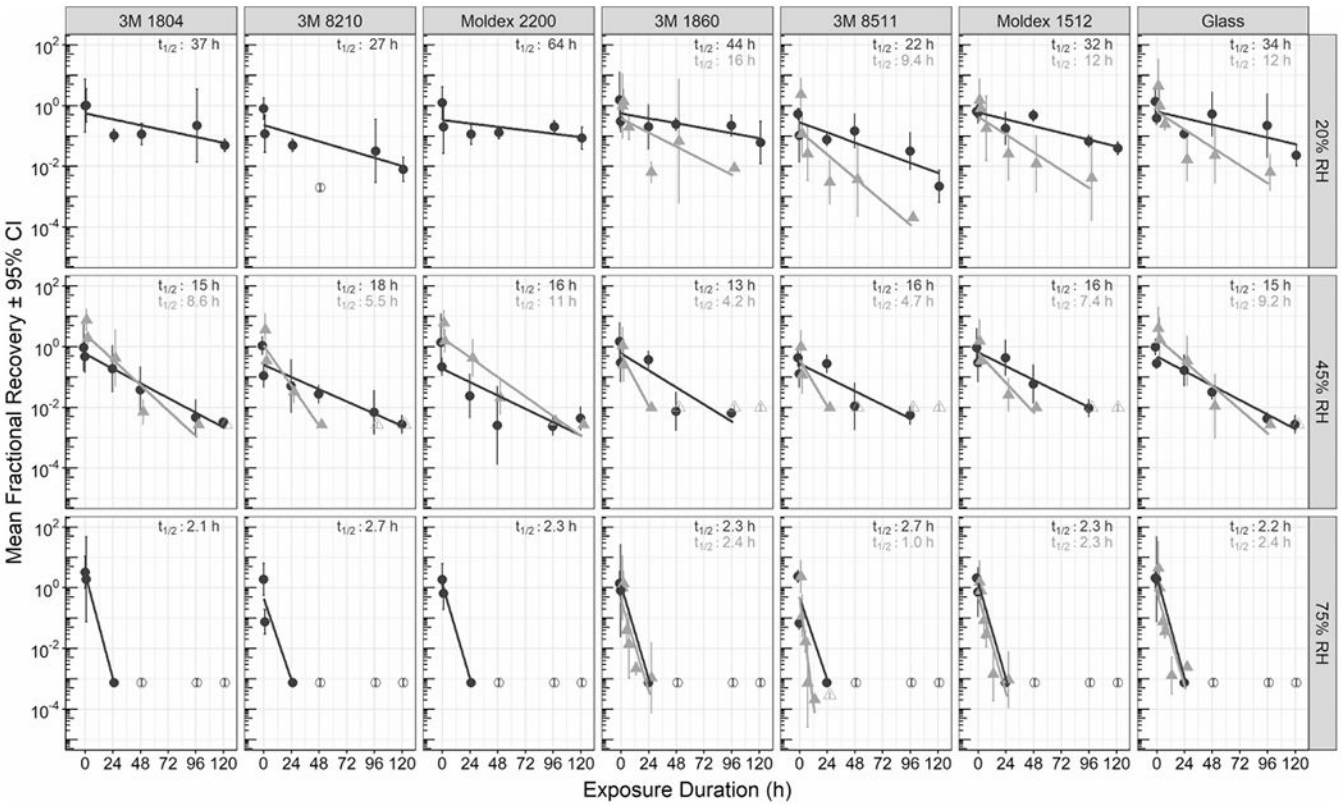

Figure 1.

SARS-CoV-2 decay on FFR models and glass slide coupons. Error bars represent one standard deviation from the mean of three replicates at each time point. The lines represent the best fit logarithmic decay curve when the virus was applied in culture medium (black circles) and saliva (grey triangles). The filled symbols represent values used in the regression model, and open symbols indicate time points at which samples were collected that were below the limit of detection and not used in half-life calculations. Data for some respirator models at 20% and 75% RH were not collected due to a lack of N95 FFRs available for research.

Table 1.

Ratio of GM half-life comparing culture medium to saliva matrix at different RH levels, with Tukey-adjusted P-values.

Relative humidity	Half-life ratio (Culture medium/saliva) (p-value)
20%	2.9 (<0.0001)
45%	2.3 (<0.0001)
75%	1.2 (0.8289)