Title: Persistence of SARS-CoV-2 on N95 filtering facepiece respirators: implications for reuse

Materials and 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 a 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 Tflasks (Sigma Cat. No. CLS3291; or equivalent) at 37^{o}C with 5% carbon dioxide. Cell lysate supernatant was harvested after approximately 72 hours incubation or when cytopathic effects (CPE) were observed on the Vero E6 cells. Resulting SARS-CoV-2 working stocks had titers of approximately 106 tissue culture infectious doses 50% [TCID50] per milliliter, and the material was stored as single-use vials at ≤-80^{o}C.

Test coupons

A total of six NIOSH Approved® filtering facepiece respirator (FFR) models were evaluated for virus persistence in this study: (1) four NIOSH Approved and FDA-cleared Surgical N95® FFRs (3M 1860, 3M 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^{o}C–22^{o}C and 30%–50% RH). The total deposition of SARS-CoV-2 was approximately 1×10^{5} TCID50 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^{o}C–22^{o}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^{o}C–22^{o}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 50-mL conical tubes containing a 10-mL extraction buffer (DMEMþpenicillin-streptomycin þ 2 to 5% fetal bovine serum). The conical tubes were agitated on a platform shaker at 200RPM for 15 min, and the extracts were 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 10-mL starting volume was concentrated to approximately 0.5 mL. Media was added to equilibrate all washed retentates to approximately 2 mL. 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^{o}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.

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