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

Introductory Information

In response to the shortage of N95® filtering facepiece respirators (FFRs) for healthcare workers during the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) issued strategies for extended use and limited reuse of N95 FFRs to conserve supply. Previously worn N95 FFRs can serve as a source of pathogens, which can be transferred to the wearer while doffing and donning a respirator when practicing reuse. To reduce the risk of self-contamination when donning and doffing reused FFRs, the CDC suggested storing FFRs for five days between uses to allow for the decay of viable pathogens including SARS-CoV-2. This study assessed the persistence of the SARS-CoV-2 strain USA-WA1/2020 on N95 FFRs under controlled storage conditions for up to five days to inform the CDC guidance.

Methods Collection

Virus propagation

- Virus used: Severe Acute Respiratory Syndrome (SARS)-Coronavirus CoV-2 strain USA-WA1/2020 (WA1), obtained from BEI Resources (Manassas, VA).
- Working stocks had titers of approximately 106 tissue-culture infectious doses 50% [TCID50] per milliliter.
- Material stored as single-use vials at ≤ -80^{o}C.

Test coupons

- Six NIOSH Approved® FFR models
    - Four NIOSH Approved and FDA-cleared Surgical N95 FFRs
        - 3M 1860 and VFlex 1804
        - Moldex 1512 and 2200
    - Two NIOSH Approved N95 FFRs
        - 3M 8210 and 8511
- Non-porous controls – glass coupons (microscope cover slides)
- Rectangular coupons (2×5 cm) taken from unused FFRs.

Coupon contamination and storage conditions

- Virus suspended in
    - Complete cell culture medium
    - Human saliva
- Ten droplets (10 µL each droplet) applied to coupons under ambient conditions (20^{o}C–22^{o}C and 30%–50% RH).
- Total deposition of SARS-CoV-2 was approximately 1×10^{5} TCID50 across the outer surface of the FFR coupon.
- Coupons were allowed to dry under ambient conditions.
- Transferred to brown paper bags and stored at 20^{o}C–22^{o}C and at 20% (15%–25%), 45% (30%–50%), and 75% (70%–75%) RH.
- 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.

    - 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.

Virus extraction and analysis

- Coupons were removed from the paper bags and placed into individual 50-mL conical tubes containing a 10-mL extraction buffer.
- 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.
    - Samples inoculated 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.
    - Observed microscopically for cytopathic effects (CPE, visible morphological changes in cell cultures caused by viral infections).
    - Used to quantitatively calculate the viral titer for each sample.
- Extraction efficiency was assessed relative to a direct spiking and quantification of the extraction medium for
    - NIOSH Approved and FDA-cleared surgical N95 FFR (3M 1860)
    - NIOSH Approved N95 FFR (3M 8511)
    - Glass control surface

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Attribution

NIOSH Approved and N95 are certification marks of the U.S. Department of Health and Human Services (HHS) registered in the United States and several international jurisdictions.

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