Room Temperature Wait and Reuse for Bioburden Reduction of SARS-CoV-2 on N95 Filtering Facepiece Respirators

Sylvia J. Smullin,1,* Branden D. Tarlow,2 and the N95DECON Consortium3

Abstract

Introduction: During a pandemic, when the supply of N95 filtering facepiece respirators (FFRs) is limited, health care workers may reuse N95 FFRs. Room temperature storage of N95 FFRs—waiting before reuse—could be a simple low-cost method to reduce severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) bioburden in such a situation. The U.S. Centers for Disease Control and Prevention specify this as a strategy for reducing self-contamination risk during a time of N95 FFR shortage.

Objective: To review the literature on persistence of SARS-CoV-2 on surfaces to assess room temperature waiting times for bioburden reduction on N95 FFRs.

Methods: The literature was searched for studies evaluating room temperature persistence of SARS-CoV-2. A 3-log decay time was extracted from published data for quantitative comparison between different studies. Studies using surgical masks and non-peer-reviewed studies that include N95 FFRs were used to draw conclusions.

Key Findings: Experimental and analytical choices vary between studies and impact the estimated 3-log decay time. There is not a clear understanding of which material properties are significant. There are no peer-reviewed studies of virus persistence on an N95 FFR.

Discussion and Conclusions: SARS-CoV-2 inactivation occurs spontaneously at room temperature. The precise timing depends on factors including humidity, temperature, and surface material. In reviewed studies, a 7-day waiting period encompasses the 3-log reduction in infectious titer of SARS-CoV-2 on specific N95 FFRs and surgical masks. Owing to variations between studies and among N95 FFR materials and room temperature conditions, it is impossible to extrapolate from these limited data to assign a precise 3-log decay time for all used N95 FFRs.

Keywords: decontamination, N95, FFR, personal protective equipment, bioburden reduction, persistence

Background

The novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) that causes coronavirus disease 2019 (COVID-19) has led to a global shortage of N95 filtering facepiece respirators (N95 FFRs, also referred to as “N95 masks”). In this document, we review the use of a room temperature storage and waiting time between uses (“wait and reuse”) as a method for bioburden reduction on N95 FFRs, with the goal of increasing the useful lifetime of N95 FFRs worn by health care providers during the COVID-19 pandemic. Effective bioburden reduction requires inactivation of the SARS-CoV-2 and maintenance of both the fit and filtration efficiency of the N95 FFR, while minimizing the risk of cross-contamination.

Room temperature storage—waiting for a minimum period before reusing an N95 FFR that is stored in a clean breathable environment at room temperature conditions—could be a simple low-cost method for bioburden reduction. This method is specified by the U.S. Centers

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for Disease Control and Prevention (CDC) as one strategy to reduce the risk of self-contamination with reuse of N95 FFRs. Enveloped RNA viruses, such as the SARS-CoV-2, eventually lose their infectious capacity at room temperature as environmental conditions lead to disruption of the virus envelope, proteins, or RNA. Viral inactivation can additionally be affected by the surface material, protein content, pH, chemicals, humidity, and the medium in which the virus is suspended. Details of how this inactivation happens are beyond the scope of this report. The precise timing and variability of this process on different materials are addressed experimentally in the studies reviewed here.

Two studies in this review tested masks identified simply as “surgical masks.” Although N95 FFRs differ from surgical masks, the N95 FFRs commonly used in medical settings, sometimes referred to as “surgical N95 FFRs,” are Food and Drug Administration (FDA) certified for functionality as surgical masks. The outer layers of both N95 FFRs and surgical masks are made from synthetic materials and the composition varies by manufacturer. Without further details of the materials of a given surgical mask, it is not possible to judge the relevance of this experiment to N95 FFRs. To present a conservative judgment of the waiting time for 3-log reduction in the bioburden of SARS-CoV-2 on N95 FFRs, results on surgical masks are included in the assessment here.

3-Log decay time and virus models

So as to have a common quantitative metric, we evaluate studies based on the time required to reach a 3-log level of viral inactivation of SARS-CoV-2. This is the time for the viral load to be reduced by a factor of 1000. This definition only considers virucidal activity and does not consider mycobactericidal or sporicidal activity.

Though this metric is inspired by guidelines of the U.S. FDA, it is different from all three tiers listed in the May 2020 FDA recommendations for sponsors seeking emergency use authorization for N95 FFR decontamination or bioburden reduction systems. Bioburden reduction as defined in this review considers virucidal activity and does not consider mycobactericidal or sporicidal activity.

Virus inactivation with time is often assumed to follow first-order kinetics (e.g., Seo et al.), which means that the number of active organisms decreases at a rate proportional to the number of organisms that exist at that moment in time. The assumption of first-order kinetics implies that there is an exponential decrease with time in the number of infectious organisms, characterized by a time constant (also called a rate constant). Moreover, this model implies that the fraction of decay in a given time interval does not depend on the size of the initial viral inoculum, that is, the time to get from 1000 active particles to 1 active particle is the same as the time to get from 5000 to 5 active particles. The rate constant for decay may change depending on the environment of the virus.

On an N95 FFR used in a medical context, virus particles may experience dramatically different local environments, leading to a broad distribution of decay rates and a deviation from the idealized exponential decay at the population level. Some experiments reviewed here show nonexponential decay, with a decay rate that becomes slower over time. Thus, extrapolating measured decays beyond the duration of the experiment may underestimate the 3-log decay time under the measured conditions.

As 3-log decay time is influenced by a number of environmental factors, it is ideally assessed through direct experiment. Moreover, the reduction in viral load may not be identical to the reduction in probability of infection. With a probabilistic dose–response model, for example, Watanabe et al. the viral infection risk decreases more slowly than the decrease of viral load. For example, if the viral load decreases by 90%, the viral infection risk decreases by <90%.

Literature Review Process

Pubmed was searched for (SARS-CoV-2) AND (surface) AND (stability) OR (persistence). Medrxiv and Biorxiv were searched for (SARS-CoV-2) AND (stability OR persistence). Related reviews were used to further discover original studies of the persistence of SARS-CoV-2 on surfaces in a room temperature environment and of human coronaviruses on personal protective equipment (PPE) under room temperature conditions.

Attempts were made to contact authors of the studies on SARS-CoV-2 for raw data and clarification of methods.

Results

Experiments that test persistence and inactivation of a virus on a surface share the same high-level steps:

1. Resuspend virus in a medium
2. Inoculate this suspension onto the material being tested
3. Wait a specific amount of time
4. Recover the virus from the material
5. Quantify number of infectious virus recovered
6. Repeat steps (2)–(5) for different wait times.

Different choices in each of these steps can lead to different reported results for how virus inactivation changes with time. Kasloff et al. included an explicit drying step between the inoculation and the waiting time; this step was not included in the other studies reviewed here. Some studies additionally fit data to a model and report a number from the fit that characterizes how the virus is inactivated over time.
In this section, we summarize recent articles on SARS-CoV-2 and earlier articles on other coronaviruses applied to materials relevant to a hospital setting. In an extensive review from the beginning of the SARS-CoV-2 pandemic, Kampf et al.\textsuperscript{11} focused on the survival of coronaviruses on surfaces and coronavirus inactivation with biocidal agents. Across experiments reviewed there, there are large variations in the reported “persistence” time for nominally the same surfaces (such as plastic). “Persistence” is undefined in Kampf et al.\textsuperscript{11} and the articles cited in that review reported varying metrics. If “persistence” is the time for the virus to reach a measurement threshold, it depends on the measurement techniques and the starting titer; the comparison across articles of the “persistence time” thus is not valid unless they use the same measurement techniques, same measurement threshold, and same starting titer.

In this study we attempt to compare all articles using the same metric of inactivation: the 3-log decay time from the start of the experiment. Even so, varying experimental and mathematical choices in the published literature led to substantial variations.

Studies are summarized in this section and the resulting 3-log decay times are quantified in Table 1. The methods in the reviewed studies are highlighted in this section to show the variations in experimental choices. Studies are listed in order of publication date. This review does not include studies published after this manuscript’s submission.

**Kasloff et al. (2020)**

In a non-peer-reviewed preprint, Kasloff et al.\textsuperscript{10} tested the stability of SARS-CoV-2 on materials used for PPE, including one N95 FFR that is also rated as a surgical mask\textsuperscript{12} and one N100 FFR that has no fluid resistance rating and is intended for industrial use.\textsuperscript{13}

Manufacturers were listed for all tested materials. As with other literature in this field, materials were characterized with the nonspecific terminology of “porous” and “nonporous” and no other material characterization was presented. Coupons, or small cutouts, of each material were sterilized before application of the experimental inoculum. The virus was prepared in a medium intended to include organic components similar to what is shed by infected people. Virus was recovered by elution in 1 mL of culture medium. Extra steps were taken to ensure recovery of low virus levels and measurements were made up to 21 days.\textsuperscript{10}

The inoculated coupon of each material was air dried for 1 h before measurements commenced.\textsuperscript{10} This drying step is unique in the studies reviewed here. Persistence time, which is the time that it took for the recovered virus to meet a measurement threshold, is the metric reported in this study. As already discussed, persistence time alone cannot be meaningfully compared across different studies unless they use the same techniques and starting titer.

The 3-log decay time given in Table 1 is the first time at which the reported titer was 3-log less than the measurement at 1 h after drying.

As given in Table 1, the 3-log decay time thus defined was at 7 days (168 h) for the N95 FFR surgical mask, as well as for the N100 particulate respirator.

In most cases, this 3-log decay time is much longer than the 1 h drying time. However, chemical gloves and cotton showed significant decrease in titer during the drying step, which challenges this particular choice for 3-log decay time. For cotton, the measurement immediately after drying showed 3-log decay from before drying.

For chemical gloves, at 4 h the measurement showed 3-log reduction from before drying. For chemical gloves and cotton, the measurement threshold was reached before 3-log reduction from the measurement after the drying step. In Table 1, the 3-log decay time is thus noted as greater than the maximum measured time.

Kasloff et al. recognized that the rapid initial drying and the extraordinarily high air flow rate in the laboratory for the duration of the experiment may not be representative of the conditions in typical medical settings and suggested that their results may be a best-case scenario.\textsuperscript{10}

**Liu et al. (2020)**

In a non-peer-reviewed preprint, Liu et al.\textsuperscript{5} tested the stability of SARS-CoV-2 on surfaces, including a surgical mask. The stability of the virus in human excreta was also tested. For surface stability, tests were at 25–27°C and relative humidity of 35%. The virus was recovered by adding 0.5 mL of viral transport medium. Data were fit to a two-phase linear model for the log of the recovered TCID\textsubscript{50}/mL against time (where TCID\textsubscript{50} is the median tissue culture infectious dose). In Table 1, the 3-log decay time is visually estimated from plots as the measurement time at which the mean virus titer was 3-log less than the measurement after the drying step. In Table 1, the 3-log decay time is thus noted as greater than the maximum measured time.

Rapid decay was observed at the beginning, with half-lives of <1 h for each material; much longer half-lives were reported for the longer time measurements.\textsuperscript{5} It is possible that the rapid decay at the beginning could reflect the drying process that would spontaneously occur for the aqueous solution at room temperature conditions.

Liu et al.\textsuperscript{5} showed data for up to 168 h for all materials. In Table 1, the maximum measured time is the time at which the measurement ended or, as judged from the plotted data, the measurement reached the threshold.

**Fischer et al. (2020)**

In a non-peer-reviewed preprint, Fischer et al.\textsuperscript{14} evaluated the stability of SARS-CoV-2 on samples of stainless steel and N95 filter material from AOSafety N9504C respirators. The virus was recovered from the material by adding 1 mL of medium. Fischer et al.\textsuperscript{14} fit the data
Table 1. Estimated 3-log decay time of coronaviruses on surfaces from studies that include medical PPE materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Virus</th>
<th>Medium^a</th>
<th>Starting titer (TCID&lt;sub&gt;50&lt;/sub&gt;/mL), inoculum</th>
<th>Environmental conditions</th>
<th>Maximum measured time (hour)^b</th>
<th>Estimated 3-log decay time (hour)^c</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal protective equipment materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N95 FFR surgical mask</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>504</td>
<td>168</td>
<td>10</td>
</tr>
<tr>
<td>N95 FFR material</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>24</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Surgical mask, inner layer</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>168</td>
<td>96</td>
<td>6</td>
</tr>
<tr>
<td>Surgical mask, outer layer</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>168</td>
<td>168</td>
<td>6</td>
</tr>
<tr>
<td>Surgical mask</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>N100 particulate respirator for industrial use</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>504</td>
<td>168</td>
<td>10</td>
</tr>
<tr>
<td>Nitrile gloves</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>168</td>
<td>168</td>
<td>10</td>
</tr>
<tr>
<td>Chemical gloves</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>96</td>
<td>&gt;96</td>
<td>10</td>
</tr>
<tr>
<td>Tyvek</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>336</td>
<td>168</td>
<td>10</td>
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<tr>
<td>Latex gloves</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>Disposable gown</td>
<td>SARS-CoV-1</td>
<td>PBS</td>
<td>10&lt;sup&gt;7&lt;/sup&gt;–10&lt;sup&gt;5&lt;/sup&gt;, 5 µL</td>
<td>Not Reported</td>
<td>48</td>
<td>70.5</td>
<td>16</td>
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<tr>
<td>Cotton gown</td>
<td>SARS-CoV-1</td>
<td>PBS</td>
<td>10&lt;sup&gt;7&lt;/sup&gt;–10&lt;sup&gt;5&lt;/sup&gt;, 5 µL</td>
<td>Not Reported</td>
<td>24</td>
<td>46.1</td>
<td>16</td>
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<td>Metals</td>
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<td></td>
<td></td>
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<td>Stainless steel</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>336</td>
<td>168</td>
<td>10</td>
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<tr>
<td>SARS-CoV-2</td>
<td></td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
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<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>168</td>
<td>168</td>
<td>6</td>
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<tr>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>168</td>
<td>5</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>96</td>
<td>56.1</td>
<td>15</td>
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<tr>
<td>SARS-CoV-1</td>
<td>VTM</td>
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<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>72</td>
<td>41.5</td>
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<tr>
<td>Copper</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;7&lt;/sup&gt;, 50 µL</td>
<td>~20°C, 30–40% RH</td>
<td>4</td>
<td>&gt;4</td>
<td>10</td>
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<tr>
<td>SARS-CoV-1</td>
<td></td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>8</td>
<td>7.7</td>
<td>15</td>
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<td>Organic materials</td>
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<td>Wood</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>48</td>
<td>6</td>
<td>6</td>
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<td>Cotton clothes</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>96.6</td>
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<td>Cloth</td>
<td>SARS-CoV-2</td>
<td>Mucin, bovine serum albumin, tryptone</td>
<td>10&lt;sup&gt;7.9&lt;/sup&gt;, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>4</td>
<td>&gt;4</td>
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<td>Glass</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>Ceramics</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>96</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>Banknote</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10&lt;sup&gt;7.8&lt;/sup&gt;, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>168</td>
<td>120</td>
<td>5</td>
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<tr>
<td>Cardboard</td>
<td>SARS-CoV-2</td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>48</td>
<td>34.5</td>
<td>15</td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td></td>
<td></td>
<td>10&lt;sup&gt;5&lt;/sup&gt;, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>24</td>
<td>5.9</td>
<td>15</td>
</tr>
</tbody>
</table>

(continued)
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Material</th>
<th>Virus</th>
<th>Medium*</th>
<th>Starting titer (TCID₅₀/mL), inoculum</th>
<th>Environmental conditions</th>
<th>Maximum measured time (hour)b</th>
<th>Estimated 3-log decay time (hour)c</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue paper</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10⁷, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>3</td>
<td>0.5</td>
<td>6</td>
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<tr>
<td>Paper</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10⁷, 5 µL</td>
<td>22°C, ~65% RH</td>
<td>3</td>
<td>&gt;0.5</td>
<td>6</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td></td>
<td>10⁶, 50 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>48</td>
<td>5</td>
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<tr>
<td>SARS-CoV-1</td>
<td>PBS</td>
<td></td>
<td>10⁻¹⁰–10⁻⁹, 5 µL</td>
<td>Not Reported</td>
<td>24</td>
<td>42.7</td>
<td>16</td>
</tr>
<tr>
<td>Plastic</td>
<td>SARS-CoV-2</td>
<td>VTM, mucin, bovine serum albumin, tryptone</td>
<td>10⁷, 10 µL</td>
<td>~20°C, 30–40% RH</td>
<td>504</td>
<td>168</td>
<td>10</td>
</tr>
<tr>
<td>(type not specified)</td>
<td>SARS-CoV-2</td>
<td>Mucin</td>
<td>10⁷, 10 µL</td>
<td>22°C, ~65% RH</td>
<td>168</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>Plastic</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10⁷, 5 µL</td>
<td>25–27°C, 35% RH</td>
<td>168</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>Plastic</td>
<td>SARS-CoV-2</td>
<td>VTM</td>
<td>10⁶, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>96</td>
<td>67.9</td>
<td>15</td>
</tr>
<tr>
<td>Plastic</td>
<td>SARS-CoV-1</td>
<td>PBS</td>
<td>10⁵, 50 µL</td>
<td>21–23°C, 40% RH</td>
<td>96</td>
<td>75.2</td>
<td>15</td>
</tr>
</tbody>
</table>

*If not named, the medium is not specified in the given study.
bThe maximum measured time is estimated differently for each reference. Details in text.
^The method by which the 3-log decay time is estimated from the published data varies by reference. Details in text.
PBS, phosphate-buffered serum; N95 FFR, N95 filtering facepiece respirator; PPE, personal protective equipment; RH, relative humidity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VTM, viral transport medium.

Chin et al. (2020) tested SARS-CoV-2 on various surfaces, including the inner and outer layer of a surgical mask. The virus was recovered from each material by soaking in 200 µL of viral transport medium for 30 min. SARS-CoV-2 was found to persist longer on non-porous materials (glass, stainless steel, and plastic) than on porous materials (paper, tissue paper, wood, and cloth). The medium used was VTM in each case.

Van Doremalen et al. (2020) tested both SARS-CoV-1 and SARS-CoV-2 on plastic, stainless steel, and cardboard, choosing an inoculum at a level relevant to samples from the human respiratory tract. For the test on cardboard, the virus was recovered by swabbing the surface and then dissolving in virus medium. Virus persistence as an aerosol was significantly less than the persistence of the virus in solution deposited on stainless steel or plastic. Measurements of aerosol persistence were also made, with samples collected on a gelatin filter that was then dissolved in virus medium.

In Table 1, the maximum time at which data were measured is the time at which the estimated titer from all three replicates first reached the threshold for detectability. In the cases wherein that was not shown on plots, it was found from the raw data at https://github.com/dylanhmorris/sars-cov-2-stability.

Although the raw data used in van Doremalen et al. (2020) for copper and cardboard did not show exponential (or even monotonic) decreases in viral load with time and thus fits are to be interpreted with caution.

Measurements of aerosol persistence were also made, with samples collected on a gelatin filter that was then dissolved in virus medium. Virus persistence as an aerosol was significantly less than the persistence of the virus in solution deposited on stainless steel or plastic. Measurements of aerosol persistence were also made, with samples collected on a gelatin filter that was then dissolved in virus medium.

Van Doremalen et al. noted that data for copper and cardboard did not show exponential (or even monotonic) decreases in viral load with time and thus fits are to be interpreted with caution. In Table 1, the maximum measured time is where the estimated mean titer across replicates was shown to reach the measurement threshold (Dylan H. Morris, personal communication, April 22, 2020). The data show the time intervals of the measurements.
beginning and a longer time constant for much longer times. It is possible that the rapid decay at the beginning could reflect the drying process that would spontaneously occur for the aqueous solution at room temperature conditions.

The 3-log decay time given in Table 1 was deduced directly from the reported raw data (rather than from the fit). Raw data were reported at 0 min, 30 min, 3 h, 6 h, 1 day, 2 days, 4 days, and 7 days. In Table 1, the 3-log decay time is the time (without interpolation) at which the mean measurement showed at least a 3-log reduction from the mean measurement at 0 min. If the detection threshold was reached before the 3-log reduction, the 3-log decay time is reported here as greater than the time at which the threshold was reached. The maximum measured time given in Table 1 is the time at which the reported data were first at the measurement threshold (undetectable) or the last time at which data were reported (even if still above threshold).

For the surgical mask on the outer layer, it is notable that there was a relatively large standard deviation (0.46) on the final measurement (mean of 2.79), which is both the measurement that defines the 3-log decay time and the longest measurement made.

The stability of the virus in viral transport medium at varying temperature was also tested in Chin et al. A linear fit on the reported log data yields 3-log decay times in medium of 2070 h at 4°C (the temperature of a household refrigerator), 167 h at 22°C (“room temperature”), and 20 h at 37°C. This implies that for a temperature change of 10°C, the 3-log decay time could change by a factor of 4–5. These data show that virus stability is highly sensitive to temperature.

Lai et al. (2005)

Lai et al. tested the earlier SARS-CoV-1, measuring its lifetime in stool and respiratory specimens as well as on paper, a disposable gown made of impervious material, and a cotton gown. To recover the virus, material was inoculated into cell culture tubes and incubated.

In the stool samples, the virus persisted longer at higher pH. In respiratory specimens, the virus persisted above the 3-log level for ~1 week at room temperature and 3 weeks at 4°C. This illustrates virus sensitivity to the local environment.

Only the times to inactivation (at the measurement threshold) for three different starting titers were reported for each material. In Table 1, the maximum measured time is the reported “time taken to inactivate” for the largest titer of 10⁶ TCID₅₀/mL.

Estimating the 3-log decay time from such minimal data requires many assumptions. Two possible approaches, both assuming that the system follows first-order kinetics, are:

1. If the system follows first-order kinetics, the time to inactivation should be a linear function of the log of the titer. With the three data points given, this linear model appears reasonable only for the case of the disposable gown. For all three materials, this method yields a 3-log decay time that is on the order of twice the longest measurement time.

2. Alternatively, if it is assumed that the threshold measurement for inactivation is 1 PFU (plaque-forming unit), a first-order kinetics model would yield a 3.5-log decay from an initial titer 10⁶ TCID₅₀/mL. Assuming that the inoculation is the same as the initial titer, the time to threshold for this titer can be used to extract a 3-log decay time. This method of estimation yields a number that is less than the total measurement time.

These two methods yield different results for each material case and the reported method in Table 1 is the larger of the two (to be conservative). These numbers are very rough estimates and they are shown merely to illustrate the need for more data and evaluation of the models used for fitting.

Sizun et al. (2000)

Sizun et al. evaluated the lifetime of human coronaviruses HCoV-229E and HCoV-OC43 when dried on surfaces and in various aqueous solutions. The difference in survival times in the different aqueous suspensions points to a challenge in doing experiments on surfaces: if survival is measured while liquid droplets of the virus suspension persist, the liquid in which the virus is suspended can impact the survival time.

To recover the virus from the materials, the material was incubated in a sonicating water bath and eluate was analyzed. The 3-log decay time cannot be extracted from the data in this article because the data are only presented as a plot on a linear (not logarithmic) scale. This article is not summarized in Table 1. For both viruses, the infectivity in the first 3 h dropped the slowest for aluminum, compared with latex gloves and sterile sponges.

Summary of results in the literature

Table 1 summarizes the mentioned tests of virus lifetimes on surfaces. Cited numbers below are mean (for frequentist analysis) or median (for Bayesian analysis) unless otherwise specified. All log values are assumed to be base 10.

Discussion

Status of federal guidance

In this unprecedented COVID-19 pandemic, due to a limited supply of N95 FFRs, the CDC has provided guidance that health care workers may practice extended use or limited reuse of N95 FFRs. The CDC’s recent guidance on reuse describes a strategy in which each health care worker is issued at least five N95 FFRs, to wear one per day, and each N95 FFR is stored in a breathable paper bag between uses. In this guidance, the health care worker is to rotate through the five N95 FFRs so that there is a waiting period of at least 5 days before reuse. In addition, the CDC has provided guidance...
to hospitals on methods for decontaminating N95 FFRs if the “wait-and-reuse” strategy is not tenable.\(^1\)

United States federal guidelines have evolved and this review may not reflect the most recent ones.

**Strategies**

NIOSH and the CDC give recommendations for N95 FFR reuse, including\(^1\)\(^{18}\):

- Storing N95 FFRs in a clean breathable container or hanging N95 FFRs between reuse.
- Discarding N95 FFRs contaminated with blood, respiratory or nasal secretions, or other bodily fluids.
- Storing N95 FFRs such that they are not damaged or deformed.
- Discarding N95 FFR that is damaged or that is hard to breathe through.

Using room temperature wait and reuse is described by the CDC as risk mitigation for extraordinary circumstances rather than as decontamination or sterilization. Room temperature storage has not been shown to acceptably reduce other microbial activity. This is reflected in the CDC recommendations\(^1\)\(^{18}\) wherein N95 FFRs stored at room temperature for reuse must be returned to their original user and should be treated as if contaminated.

Given the unknowns in using room temperature wait and reuse as a method of bioburden reduction, proper donning, doffing, and hand hygiene are critical for reducing risk and are part of the NIOSH/CDC recommendations.\(^18\) For example, Brady et al.\(^19\) showed that improper doffing of an N95 FFR, even without reuse, can lead to higher contamination to hands than proper doffing and subsequent reuse.

If N95 FFRs are stored in a clean breathable environment at room temperature between uses, without deformation or crushing, a primary risk to integrity is the degradation in fit over multiple donnings and doffings. This was studied in Bergman, et al.\(^20\) For some N95 FFR models, fit was found to be unacceptable after 5 don–doff cycles, whereas others maintained fit for >15 don–doff cycles.\(^20\) A user seal check before each reuse can help address this risk, and is recommended by the CDC.\(^1\) In the absence of guidance from a manufacturer for a specific N95 FFR model, the CDC recommends no more than five donnings per N95 FFR device.\(^1\)

As already summarized, virus persistence is expected to be much greater at lower temperatures. The presented studies all use moderate humidity; higher or lower humidity may change the virus inactivation time.\(^3\)\(^,\)\(^4\)\(^,\)\(^21\)

**Primary risks and unknowns**

Enveloped RNA viruses such as SARS-CoV-2 tend to be more rapidly inactivated at room temperature than other clinically relevant pathogens that could coinoculate an N95 FFR such as mycobacterium, antibiotic resistant bacteria, bacterial spores, or other pathogens. An adequate wait time that inactivates SARS-CoV-2 may not inactivate other common pathogens. Analysis of an appropriate waiting time for inactivation of other pathogens is beyond the scope of this report.

The existing literature leaves several gaps that necessitate judgment in choosing a time period for bioburden reduction of SARS-CoV-2. In two peer-reviewed studies on SARS-CoV-2,\(^6\)\(^,\)\(^15\) the environmental conditions (in terms of temperature and humidity) were similar. Stainless steel and plastic were tested in both of these articles, without details on the materials or the surface finish. When the results from these two references are compared using the same gauge of persistence—a 3-log decay in virus level—there are discrepancies.

The 3-log decay time of SARS-CoV-2 on stainless steel was between 4 and 7 days in Chin et al.\(^6\) and \(~2.3\) days in van Doremalen et al.\(^15\)

Chin et al.\(^6\) showed a 3-log decay time on plastic of 2 days and van Doremalen et al.\(^15\) showed that of 68 h \((\sim3\) days).

The discrepancies between Chin et al.\(^6\) and van Doremalen et al.\(^15\) cannot be reconciled from the available data. This presents a large uncertainty on enumerating an appropriate waiting period for bioburden reduction.

Van Doremalen et al.\(^15\) compared SARS-CoV-1 and SARS-CoV-2, which illustrates the uncertainty that may be incurred by extrapolating from the results of one virus to another virus. Across the five tested cases, these differences (median, extrapolated to 3-log decay) range from \(~1\) h (for the short life of aerosols) to \(~1\) day (for cardboard).

Van Doremalen et al.\(^15\) gave 95% confidence intervals for each result. These confidence intervals for the SARS-CoV-2, again extrapolating to the 3-log decay time, were \(~1\) day in most cases.

Together, these results point to the high uncertainty in the published data and the need for more experiments with clearly specified materials and methods and more variables (such as starting titer) that are varied.

In this review, we enumerate some of the experimental and modeling choices, assumptions, and possible variability in implementation that leave uncertainty.

1. The assumption of first-order kinetics is a mathematically convenient assumption that has proven to be effective.\(^22\) However, it is not always the best model for fitting all of the data. Indeed, in Chin et al.\(^6\) and Liu, et al.,\(^5\) the data for survival of SARS-CoV-2 on different surfaces were fit to a model with a different time constant in the first hour than for the duration of the experiment. None of the studied articles give assessments of the quality of one model versus another. There are other models for virus kinetics: in Seo et al.,\(^8\) a Weibull model was found to fit the data better.
for murine norovirus. If the first-order kinetics model is not the right model, then the time for each additional log of decay might be longer than for the previous log of decay.

2) Model fits may not be appropriate for 3-log decay time if the time of the experiment is much shorter than the extrapolated 3-log decay time. For example, in Kasloff et al., the measurement threshold was met before 3-log decay was demonstrated after drying for chemical gloves and cotton clothes.

3) It is expected that the time constant, even in a first-order model, will depend on environmental conditions. Vejerano and Marr, for example, showed that the relative humidity determines the evaporation rate of a droplet and argued that virus survival is impacted by the microenvironment of this evaporating droplet. Other viruses have been shown to persist longer at extreme values of humidity than at moderate values. Temperature has a dramatic impact on virus survival times as shown, for example, in Seo et al. for the norovirus, in Chan et al. for SARS-CoV-1, and in Fischer et al. for SARS-CoV-2. User conditions may be highly variable and different from the controlled environment of a laboratory. For example, storage at temperatures colder than tested (e.g., in an unheated cabinet, basement, or vehicle where temperature falls <22°C) could substantially extend the life of the virus beyond what is described.

4) Even with the same model, how the data are analyzed can matter. Peleg and Cole gave one example for how choices in fitting data to a model can matter. Incidentally, the data for stainless steel in van Doremalen et al. and in Fischer et al. are reported to be the same data, with a difference in titer inference methods (Dylan H. Morris, personal communication, April 19, 2020). That yielded a difference of ~10 h in the reported 3-log decay time.

5) Experiments in the literature used different viruses, different media, and different methods for recovering the virus from the surface; all of these may impact the results.

6) Virus inactivation times vary widely across different materials and the reviewed articles give no fundamental understanding of why a certain material might promote longer or shorter virus survival times. This creates a challenge in extrapolating from data on one material to inactivation times for another material. For example, the material of the N95 FFR in Fischer et al. may be different, from the point of view of virus inactivation, from the material used in the N95 FFRs marketed for use in medical settings. The brand of surgical mask used in Chin et al. was not specified and it is unknown how this material compares with a given N95 FFR. In general, N95 FFRs are fabricated from layers of differently textured polypropylene, and layers sometimes include other materials such as polyester. Surgical N95 FFRs (which are FDA certified for additional functionality as surgical masks, and which are typical in health care settings) commonly have an additional hydrophobic outer layer, whereas non-surgical N95 FFRs may have a hydrophilic outer layer. These material differences are another source of uncertainty in the data.

In this report, we have used 3-log decay time as a standard way of comparing across different experiments. The initial infectious viral load will greatly impact what infection risk remains after waiting a given period of time.

Conclusions

SARS-CoV-2 and other enveloped viruses survive for a limited time on surfaces at room temperature; the precise time period needed for satisfactory inactivation depends on a number of environmental variables.

Although there are many modeling assumptions that go into the experiments as well as variability in the tested environments, across the literature surveyed here, there are qualitative conclusions that can be drawn:

- Coronaviruses, including SARS-CoV-2, generally have been shown to live longer on surfaces that are qualitatively described as smooth or nonporous than on surfaces described as rough or porous. A notable exception from experiments is copper, which yields a very short lifetime for SARS-CoV-2.
- There is a need for a better understanding of which material properties determine virus lifetime on a surface. Without that, it is a challenge to extrapolate experimental results from one material to another.
- The risk of exposure to SARS-CoV-2 virus from an N95 FFR stored individually in a clean breathable room temperature environment goes down the longer one waits before reusing the N95 FFR.

An N95 FFR stored in a moderately humid room temperature environment (22°C, 40–65% relative humidity) will eventually achieve 99.9% reduction in viral load after some waiting period. The most relevant studies show significant variation, yielding estimates between <1 day and ~1 week required for the 3-log decay time for SARS-CoV-2 on N95 FFRs or surgical masks. Only two of those studies, neither of which were peer reviewed, specify an N95 FFR. This is an area where new experimentation is urgently needed to provide more clear actionable information.

The time needed to reduce infection risk of an enveloped RNA virus to an acceptable level depends on the amount that is originally deposited, the threshold for infectiousness, and the environmental conditions including...
temperature, humidity, surface type, and the presence of other agents including proteins and salt. Critically, cooler temperatures will extend the life of SARS-CoV-2.

Given the sensitivity of the virus to material and local environment, there are not enough published data to make a precise conclusion that encompasses all N95 FFR models in a range of room temperature conditions. A 7-day period encompasses the observed 3-log decay time in the reviewed studies on specific N95 FFRs and surgical masks in specific environments. However, it is impossible to extrapolate from these limited data to assign a precise 3-log decay time for any specific environment and used N95 FFR.

More studies are needed to have higher confidence in recommendations, especially considering the range of room temperature conditions that exist in health care situations and the range of materials used for different models of N95 FFRs. It would be especially useful to have further studies that encompass measurement times well beyond the 3-log decay time, that test at different humidities and temperatures, and that prepare the virus in a suspension of a medium that is similar to human mucus.

Understanding the real-world viral loads that are deposited on an N95 FFR after close contact with an infected patient or performing an aerosol-generating procedure will help make better recommendations on waiting periods. The bioburden may fall below the infectious threshold faster if realistic viral burdens are below what was used in the reviewed experiments.

With a waiting time that is chosen merely for SARS-CoV-2 bioburden reduction, room temperature wait and reuse may not decontaminate the N95 FFR against other pathogens or infectious agents. Irrespective of the waiting time that is chosen, proper donning and doffing of the N95 FFR and hand hygiene are critical for safer reuse.

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