# Technical Report for Room Temperature Storage of N95 FFR for Bioburden Reduction and Reuse

Much of the available literature on decontamination of N95 FFRs is a result of recent efforts to relieve the shortage of N95 FFRs during the SARS-CoV-2 outbreak. Because of this, some of the research cited in this document is not yet peer reviewed. **For clarity, wherever non-peer-reviewed results are cited in this report, the citation is preceded by a "*".**

## Executive Summary

Room temperature storage—simply waiting for a minimum period before re-using an N95 FFR that is stored in a clean, breathable environment at moderately humid, room temperature conditions—is potentially the simplest and lowest cost viral inactivation method. In addressing the global shortage of N95 Filtering Facepiece Respirators (N95 FFRs), clean, room temperature storage for 5 days is a first recommendation for treating N95 FFRs between re-use in a healthcare setting (CDC, 2020b). However, published data on the lifetime of SARS-CoV-2 on surfaces are sparse, making it difficult to draw conclusions about decontamination on N95 FFRs.

In this report, we analyze recent literature, comparing results on a common quantitative basis. **This is an area where new, peer-reviewed experimentation is urgently needed to provide more clear, actionable advice.**

**For an N95 FFR that is stored individually in a clean and breathable container at room temperature, a 7 day waiting period before reuse is expected to significantly decrease risk of exposure to SARS-CoV-2 via the N95 FFR.** With additional precautions, such as individual storage in a clean, breathable container, user seal checks, hand hygiene, and proper donning and doffing, this waiting time can significantly reduce SARS-CoV-2 infection risk with re-use of N95 FFRs. This method will not protect against bacterial or fungal infection.

Viral inactivation is sensitive to temperature and humidity. **Storage at temperatures below 22°C or at very low or very high humidities is expected to significantly increase the acceptable waiting period. More data are needed to quantify these effects.**

## 1. Overview

The novel coronavirus (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 as a method to reduce the bioburden on N95 FFRs with the goal of increasing the useful lifetime of N95 FFRs worn by healthcare providers during the COVID-19 pandemic. Effective bioburden reduction requires inactivation of the SARS-CoV-2 virus and maintenance of both the fit and filtration efficiency of the N95 FFR while minimizing the risk of cross-contamination.

Room temperature storage—simply waiting for a minimum period before re-using an N95 FFR that is stored in a clean, breathable environment at room temperature conditions—is

potentially the simplest and lowest cost viral inactivation method. Enveloped RNA viruses, such as the coronavirus SARS-CoV-2, eventually lose their infectious capacity at room temperature. The precise timing and variability of this process are addressed experimentally in a number of studies that we review in this document.

Here, we evaluate studies based on the time required to reach a 3-log level of viral inactivation. This 3-log decay time is the time for the viral load to be reduced by a factor of 1000, which is the target level of reduction identified in FDA guidance documents for decontamination (FDA, 2020a). Following (FDA, 2020b), we call this 'bioburden reduction'. If a different standard were chosen for decontamination or bioburden reduction, this time would be longer. For example, the time to reach 6-log decay is expected to be at least twice the time needed for 3-log decay.

The reduction in viral load may not be identical to the reduction in probability of infection. With a probabilistic dose-response model, e.g. (Watanabe et al., 2010), the viral infection risk decreases more slowly than the viral load decreases. For example, if the viral load decreases by 90%, the viral infection risk decreases by <90%.

In this report, we survey the existing literature, highlighting assumptions that are required to interpret the data and clear qualitative conclusions that can be drawn from multiple studies. A waiting period of 7 days (168 hr) encompasses the available experimental data on bioburden reduction (as defined by 3-log decay on the sample mean) of a single N95 FFR and of a surgical mask. **This is an area where new experimentation is urgently needed to provide more clear, actionable advice.**

**Reusing the same N95 FFR within a day (i.e. at next shift) is not expected to, in general, allow sufficient time for viral inactivation and is not recommended if the N95 FFR is not also decontaminated via another effective method.** Given the sensitivity of the virus to material and local environment, we do not have enough data to make a precise recommendation that encompasses all N95 FFR models in reasonable room temperature conditions. **For an N95 FFR that is stored individually in a clean and breathable environment at room temperature, a 7 day waiting period before reuse is expected to significantly decrease risk of exposure to SARS-CoV-2 via the N95 FFR.**

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. For example, storage at temperatures colder than tested (e.g. in an unheated cabinet, basement or vehicle where temperature falls below 22°C) could substantially extend the life of the virus beyond what is described.**

## 2. Status of Federal Guidance

In this unprecedented COVID-19 pandemic, due to a limited supply of N95 FFRs, the Centers for Disease Control and Prevention (CDC) have provided guidance that healthcare workers can practice extended use or limited reuse of N95 FFRs (CDC, 2020a). In addition, the CDC has provided guidance to hospitals on methods for decontaminating N95 FFRs during a crisis (CDC, 2020b).

The Occupational Safety and Health Administration (OSHA) states that facial makeup or other barriers should not be present during respirator use (OSHA, n.d.). Emergency use authorizations (EUAs) that the FDA has granted for N95 FFR decontamination during the COVID-19 pandemic also stipulate that cosmetics not be present on respirators sent for decontamination (*Instructions for Healthcare Personnel: Preparation of Compatible N95 Respirators for Decontamination by the Battelle Memorial Institute Using the Battelle Decontamination System*, 2020).

After decontamination, the CDC recommends that a 'user seal check' is performed when the respirator is donned to ensure adequate seal (CDC, 2020b). A user seal check after every decontamination cycle is especially important because there is evidence that the fit factor of N95 respirators decreases with numerous don/doffs (Bergman et al., 2012).

Per FDA guidelines for N95 FFR decontamination EUAs, virucidal decontamination requires ≥ 3-log reduction (corresponding to a 99.9% reduction) in viral activity (FDA, 2020a). Based on this guideline, we describe a process as sufficiently "inactivating" or "decontaminating" only when it leads to a ≥ 3-log reduction in viral activity. **In this version of this report, we refer to the 3-log decay as "bioburden reduction" and unless otherwise specified, bioburden reduction thus defined considers virucidal activity and does *not* consider mycobactericidal or sporicidal activity**, for which the FDA has other guidelines (FDA, 2020a). **N95 FFR decontamination processes for SARS-CoV-2 do not necessarily result in sterilization (killing of all microorganisms) of the N95 FFR**. Moreover, this definition of bioburden is different from all three tiers listed in the more recent FDA recommendations for sponsors seeking Emergency Use Authorization for decontamination of N95s (FDA, 2020b).

A first recommendation from the CDC's recent guidance on reuse (CDC, 2020b) is for each healthcare worker to be issued at least five N95 FFRs, to wear one per day, and to store each in a breathable paper bag between uses. The healthcare worker is to rotate through the five N95 FFRs so that there is a waiting period of at least 5 days before reuse. In this guidance from the CDC, healthcare workers are instructed to treat N95 FFRs as if they are still contaminated.

Any new methods for decontamination or bioburden reduction should be verified through organizations' internal processes, which may include FDA clearance prior to implementation. Please refer to current CDC guidelines that are updated regularly, as well as N95DECON's Full Legal Disclaimer.

## 3. Mechanism

For N95 FFRs that are stored at room temperature, environmental conditions can eventually lead to disruption of the virus envelope, proteins, or RNA. Viral inactivation can additionally be affected by the surface material, protein content, pH, chemicals, and the medium in which the virus is prepared (e.g. Chan et al., 2011; Coulliette et al., 2013; Firquet et al., 2015). Details of how this inactivation happens are beyond the scope of this report.

Virus inactivation with time is often assumed to follow first-order kinetics (e.g. Seo et al., 2012), which means that the number of active organisms decreases at a rate proportional to the number 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, i.e. the time to get from 1000 active particles to 1 active particle is the same as the time to get from 5000 to 5.

On a real N95 FFR, different 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 observe non-exponential decay, a characteristic feature of which is that the decay rate becomes slower over time. Because of this, caution is needed in extrapolating measured decays beyond the measurement interval.

Thus, 3-log decay time is influenced by a number of environmental factors and ideally should be assessed via direct experiment.

## 4. Potential for SARS-CoV-2 Inactivation

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

(1) Re-suspend the 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 the amount of infectious virus recovered
(6) Repeat in parallel steps (2)–(5) for different wait times
(7) Fit thedata to a model
(8) From the model fit, report a number that characterizes how the virus is inactivated over time.

Different choices in each of these steps can lead to different reported results for how virus inactivation changes with time.

In this section, we summarize the recent papers that studied SARS-CoV-2 and two earlier papers that studied other coronaviruses applied to materials relevant to a hospital setting. Due to the paucity of data, we include non-peer-reviewed preprints with the recognition that these papers may not meet the standard of peer review.

In an extensive review prior to the SARS-CoV-2 pandemic, (Kampf et al., 2020) focused on the survival of coronaviruses on surfaces and coronavirus inactivation with biocidal agents. Across the reviewed experiments in (Kampf et al., 2020), there are large variations in the reported “persistence” time. For example, the reported times for the un-defined “persistence” of coronaviruses on plastic spans more than an order of magnitude in time, from hours to days. Part of this variation is likely due to what “persistence” time means; it is undefined in (Kampf et al., 2020) and the papers cited in that review report varying metrics.

In contrast, here we attempt to compare all papers using the same metric of inactivation: the 3-log decay time. We focus on medical materials and SARS-CoV-2 experiments. Even when making the comparison of the 3-log time across different studies,

varying experimental and mathematical choices in the published literature can lead to substantial variations.

Studies are summarized in this section and the resulting 3-log decay times are quantified in the data tables below. Methods are highlighted in this section to show the variations in experimental choices.

In a non-peer-reviewed preprint, **(*Liu et al., 2020)** tested the stability of SARS-CoV-2 on surfaces and in human excreta. For surface stability, tests were at 25–27℃ 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 $TCID_{50}$/mL against time (where $TCID_{50}$ is the median tissue culture infectious dose). From plotted data, the 3-log decay time reported in Table 1 was estimated as the measurement time at which the mean virus titre was first more than 3-log less than the starting titre. These estimates are rough as they were visually estimated from plots. Authors have not responded to multiple requests for the raw data.

In these experiments, rapid decay was observed at the beginning, with half-lives of less than 1 hr for each material; much longer half-lives were reported for the longer-time measurements.

(*Liu et al., 2020) tested a surgical mask as one of the materials. While N95 FFRs differ from surgical masks, the N95 FFRs commonly used in medical settings, sometimes referred to as “surgical N95 FFRs,” are certified for functionality as surgical masks. Without further details of the material, it is not possible to judge the relevance of this experiment to N95 FFRs. A conservative assessment of the appropriate waiting time for an N95 FFR could encompass this result.

In Table 1, the maximum measured time for (*Liu et al., 2020) is given as 168 hr for all materials. The measurement threshold appears, based on plots, to have been reached as early as 120 hrs only for the case of cotton clothes.

In a non-peer-reviewed preprint, **(*Fischer et al., 2020)** 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. The data were fit using Bayesian regression assuming exponential decay. The 3-log decay time from this study in Table 1 is the reported median “time to one thousandth” from Bayesian regression. For the N95 FFR, that time was 13 hr, with a 95% confidence interval spanning 11–15 hr.

In Table 1, the maximum measured time is where the estimated mean titre across replicates was shown in plotted data to reach the measurement threshold (Dylan H. Morris, personal communication, April 22, 2020). The data at https://github.com/dylanhmorris/n95-decontamination show the time intervals used.

**(van Doremalen et al., 2020)** tested both SARS-CoV-1 and SARS-CoV-2, 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 adding 1 mL DMEM. For other surfaces, the virus was recovered by adding 1 mL DMEM. Data show that the viruses

persisted longer on plastic and stainless steel than on cardboard or copper. Virus persistence as an aerosol was also significantly less than on stainless steel or plastic.

It is noted in this paper 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.

For Table 1, the maximum time at which data were measured is the time at which the estimated titre from all three replicates first reaches the threshold for detectability. In almost all cases this time is shown on the plots. The raw data are on Github at https://github.com/dylanhmorris/sars-cov-2-stability.

Though the raw data in (van Doremalen et al., 2020) for stainless steel are the same as in (*Fischer et al., 2020), the two papers used different titre inference methods (Dylan H. Morris, personal communication, April 19, 2020). To estimate the 3-log decay time, we multiplied the median reported half-life by 9.966.

**(Chin et al., 2020)** tested SARS-CoV-2 on various surfaces, including the inner and outer layer of a surgical mask. As described above for (*Liu et al., 2020), without further information about the material used by (Chin et al., 2020), it is not possible to judge the relevance of this experiment to N95 FFRs. Authors did not respond to a request for more information. **In the interest of being conservative, we have included this result for a surgical mask in our overall assessment.**

The stability of the virus in viral transport medium at varying temperatures was also tested in (Chin et al., 2020). We used a simple linear fit on the reported log data to extract 3-log decay times of 2070 hr at 4℃ (the temperature of a household refrigerator), 167 hr at 22℃ (‘room temperature’), and 20 hr at 37℃. This implies that for a temperature change of 10℃, the 3-log decay time could change by a factor of 4 or 5. Though these data were taken for virus in solution, not on a surface, they show that virus stability is highly sensitive to temperature.

In (Chin et al., 2020), the virus was recovered from each material by soaking in 200 uL of viral transport medium for 30 min. SARS-CoV-2 was found to remain infectious longer on non-porous materials (glass, stainless steel, plastic) than on porous materials (paper, tissue paper, wood, cloth).

Data were fit to a bi-phasic model, instead of to a simple exponential model. Data were fit assuming that the kinetics follow exponential decay with one time constant at the beginning and a longer time constant for much longer times. Thus, the reported model fits cannot be directly compared with model fits from other papers that assume exponential decay with one time constant.

Instead, the 3-log decay time given in Table 1 was deduced directly from the raw data reported at 0 min, 30 min, 3 hr, 6 hr, 1 day, 2 days, 4 days, 7 days. In Table 1, the 3-log decay time is the time (without interpolation) at which the mean measurement showed 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 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.

**(Lai et al., 2005)** tested the earlier SARS-CoV-1 virus, measuring its lifetime in stool and respiratory specimens as well as on paper (from a laboratory request form), 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 about a 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 titres were reported for each material. In Table 1, the maximum measured time is the reported "time taken to inactivate" for the largest titre of $10^6$ $TCID_{50}$/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 titre. With the three data points given, this fit is good 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 titre $10^6$ $TCID_{50}$/mL. Assuming that the inoculation is the same as the initial titre, the time to threshold for this titre 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.

Both methods were applied to the data from this publication. These two methods yield different results for each material case and the reported one 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)** 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 there are persistent droplets, 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 is analyzed. The 3-log decay time cannot be extracted from the data in this paper because the data are only presented as a plot on a linear (not logarithmic) scale and thus this paper is not summarized in Table 1. For both viruses, the infectivity in the first 3 hr dropped the slowest for aluminum, compared to latex gloves and sterile sponges.

## 5. Data Summary Tables

Table 1 summarizes the above-mentioned tests of virus lifetime on surfaces. Many of these papers include other experiments (such as lifetime in stool and respiratory specimens or responsiveness to disinfectants); those additional results are not summarized here. 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.

*Table 1*

| ***Material*** | ***Virus (medium[a])*** | ***Starting Titre/ Inoculum*** | ***Environmental Conditions*** | ***Maximum measured time (hr)[b]*** | ***Estimated 3-log decay time (hr)[c]*** | ***Ref*** |
|---|---|---|---|---|---|---|
| ***Personal Protective Equipment Materials*** | | | | | | |
| N95 FFR material | SARS-CoV-2 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23°C, 40% RH | 24 | 13 | (*Fischer et al., 2020) |
| Surgical mask, inner layer | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22°C, ~65% RH | 168 | 96 | (Chin et al., 2020) |
| Surgical mask, outer layer | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22°C, ~65% RH | 168 | 168 | (Chin et al., 2020) |
| Surgical mask | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27°C, 35% RH | 168 | 120 | (*Liu et al., 2020) |
| Latex gloves | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27°C, 35% RH | 168 | 120 | (*Liu et al., 2020) |
| Disposable gown | SARS-CoV-1 (PBS) | $10^4$–$10^6$ $TCID_{50}$/mL; apply 5 µL | *Not Reported* | 48 | 70.5 | (Lai et al., 2005) |
| Cotton gown | SARS-CoV-1 (PBS) | $10^4$–$10^6$ $TCID_{50}$/mL; apply 5 µL | *Not Reported* | 24 | 46.1 | (Lai et al., 2005) |
| ***Metals*** | | | | | | |
| Stainless steel | SARS-CoV-2 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23°C, 40% RH | 48 | 48.2 | (*Fischer et al., 2020) |
| | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22°C, ~65% RH | 168 | 168 | (Chin et al., 2020) |
| | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27°C, 35% RH | 168 | 168 | (*Liu et al., 2020) |
| | SARS-CoV-2 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23°C, 40% RH | 96 | 56.1 | (van Doremalen et al., 2020) |
| | SARS-CoV-1 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23°C, 40% RH | 72 | 41.5 | (van Doremalen et al., 2020) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Copper | SARS-CoV-2 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 8 | 7.7 | (van Doremalen et al., 2020) |
| | SARS-CoV-1 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 24 | 14.9 | (van Doremalen et al., 2020) |
| ***Organic Materials*** | | | | | | |
| Wood | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 48 | 6 | (Chin et al., 2020) |
| | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 96 | (*Liu et al., 2020) |
| Cotton Clothes | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 72 | (*Liu et al., 2020) |
| Cloth | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 48 | >24 | (Chin et al., 2020) |
| ***Other*** | | | | | | |
| Glass | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 120 | (*Liu et al., 2020) |
| | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 96 | 48 | (Chin et al., 2020) |
| Ceramics | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 120 | (*Liu et al., 2020) |
| Banknote | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 96 | 48 | (Chin et al., 2020) |
| ***Paper-based Materials*** | | | | | | |
| Cardboard | SARS-CoV-2 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 48 | 34.5 | (van Doremalen et al., 2020) |
| | SARS-CoV-1 | $10^5$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 24 | 5.9 | (van Doremalen et al., 2020) |
| Tissue paper | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 3 | 0.5 | (Chin et al., 2020) |
| Paper | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 3 | >0.5 | (Chin et al., 2020) |
| | SARS-CoV-2 | $10^6$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 48 | (*Liu et al., 2020) |
| | SARS-CoV-1 (PBS) | $10^4$–$10^6$ $TCID_{50}$/mL; apply 5 µL | *Not Reported* | 24 | 42.7 | (Lai et al., 2005) |
| ***Plastic*** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Plastic (type not specified) | SARS-CoV-2 (VTM) | $10^{7.8}$ $TCID_{50}$/mL; apply 5 µL | 22℃, ~65% RH | 168 | 48 | (Chin et al., 2020) |
| | SARS-CoV-2 | $10^{6}$ $TCID_{50}$/mL; apply 50 µL | 25–27℃, 35% RH | 168 | 120 | (*Liu et al., 2020) |
| | SARS-CoV-2 | $10^{5}$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 96 | 67.9 | (van Doremalen et al., 2020) |
| | SARS-CoV-1 | $10^{5}$ $TCID_{50}$/mL; apply 50 µL | 21–23℃, 40% RH | 96 | 75.2 | (van Doremalen et al., 2020) |

[a]VTM=viral transport medium. PBS=phosphate buffered serum. If not named, the medium was not specified in the given study.
[b]The maximum measured time is estimated differently for each reference. See details in the text above.
[c]The method by which the 3-log decay time is estimated from the published data varies by reference. See details in the text above.

## 6. Integrity of N95 Filtering Facepiece Respirators

If N95 FFRs are stored in a clean, vented environment at *room temperature* between uses, a primary risk to integrity is the degradation in fit over multiple donnings and doffings. For some N95 FFR models, fit was found to be unacceptable after 5 don/doff cycles, while others maintained fit for >15 don/doff cycles (Bergman et al., 2012). Storage conditions should not deform or crush the N95 FFR.

Furthermore, risk with reuse can be reduced by discarding N95 FFRs with visible blood, hair, soiling with facial cosmetics, or damage (CDC, 2020a; OSHA, n.d.).

## 7. Strategies

NIOSH and the CDC give recommendations for N95 FFR reuse, including storage in a clean, breathable container or hanging N95 FFRs between reuse (CDC, 2020a). N95 FFRs contaminated with blood, respiratory or nasal secretions, or other bodily fluids should be discarded (CDC, 2020a). N95 FFRs should be re-used only by the original user.

Using room temperature storage as a method for virus inactivation is viewed as risk mitigation for extraordinary circumstances rather than as complete decontamination or sterilization. Risk is further mitigated if a re-used N95 FFR is treated, in terms of hand hygiene for example (CDC, 2020a), as if the N95 FFR might still be contaminated, as recommended in (CDC, 2020b).

Given the unknowns in using room temperature storage for bioburden reduction, proper donning, doffing, and hand hygiene are critical for reducing risk. (Brady et al., 2017) shows that improper doffing of an N95 FFR can lead to higher contamination from N95 FFR to hands than proper doffing and reuse (without decontamination). Hand hygiene is another part of the recent NIOSH / CDC recommendations (CDC, 2020a).

As summarized above, virus persistence is expected to be much greater at lower temperatures. The presented studies all use moderate humidity; based on studies of other viruses, there is evidence that higher or lower humidity may change virus inactivation time (Chan et al., 2011; Coulliette et al., 2013; Lin & Marr, 2020).

After the waiting period and before re-use, physical inspection and a 'user seal check', as recommended by the CDC, should be performed to ensure N95 FFR integrity and adequate seal (CDC, 2020b).

## 8. 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 co-inoculate 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 common pathogens is currently 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 via room temperature storage. This is illustrated by the range of 3-log decay times across reviewed papers for the virus on stainless steel: (Chin et al., 2020) and (*Liu et al., 2020) showed 168 hr, (van Doremalen et al., 2020) showed 56 hr and from the same data (*Fischer et al., 2020) reported 48 hr. The discrepancy between (Chin et al., 2020) and (van Doremalen et al., 2020) cannot be reconciled from the available data and it presents a large uncertainty on enumerating an appropriate waiting period for bioburden reduction.

Here, we enumerate some of the experimental and modeling choices and assumptions that leave uncertainty.

(1) The assumption of first-order kinetics is a mathematically-convenient assumption that has proven to be effective (Peleg & Cole, 1998). However, it is not always the best model for fitting the data. Indeed, in (Chin et al., 2020) and (*Liu et al., 2020), the data for survival of CoV-2 on different surfaces were fit to a model where the time constant in the first-order kinetics is different in the first hour than over the duration of the experiment. None of the studied papers give assessments of the quality of one model compared to another. As an example of a different model for virus kinetics, in (Seo et al., 2012), a Weibull model was found to fit the data better for murine norovirus.

(2) 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.

(3) Model fits may not be appropriate for 3-log decay time if the assessed time is much longer than the time of the experiment. In (Chin et al., 2020), 7 days is the longest measurement time used. In (van Doremalen et al., 2020), the experiments were less than 100 hr in duration at maximum and extrapolation to the 3-log decay time may be longer than what was measured.

(4) It is expected that the time constant, even in a first-order model, will depend on environmental conditions. In (Vejerano & Marr, 2018), for example, it was shown that the relative humidity determines the evaporation rate of a droplet and it was argued that virus survival is impacted by the micro-environment of this evaporating droplet. Other viruses have been shown to persist longer at extreme values of humidity than at moderate values (Lin & Marr, 2020). Temperature has been shown to have a dramatic

impact on virus survival times, for example, in (Seo et al., 2012) for the norovirus, in (Chan et al., 2011) for SARS-CoV-1, and in (Chin et al., 2020) for SARS-CoV-2. User conditions may be highly variable and different from the controlled environment of a lab. For example, storage at temperatures colder than tested (e.g. in an unheated cabinet, basement or vehicle where temperature falls below 22°C) could substantially extend the life of the virus beyond what is described here.

(5) Even with the same model, how the data are analyzed can matter. (Peleg & Cole, 1998) 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., 2020) and in (*Fischer et al., 2020) 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 almost 10 hr in the 3-log decay time.

(6) Experiments in the literature used different viruses, different media, and different methods for recovering the virus from the surface and all of these can impact the results. (Chin et al., 2020) used viral medium and the other two SARS-CoV-2 studies did not report what medium is used. The inactivation time for a virus protected by mucus, for example, could be different.

(7) Virus inactivation times vary widely across different materials, as shown in the reviewed papers. The reviewed papers 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., 2020) may be different, from the point of view of virus inactivation, from the material used in the N95 FFRs that are marketed for use in medical settings. The surgical mask used in (Chin et al., 2020) is not specified and it is unknown how this material compares to a given N95 FFR. In general, N95 FFRs are fabricated from layers of differently-textured polypropylene, and layers sometimes include other materials like polyester. They have hydrophobic and hydrophilic layers varying by model. Surgical N95 FFRs, typical in healthcare settings, commonly have an additional hydrophobic outer layer, while non-surgical N95 FFRs may have a hydrophilic outer layer (Viscusi et al., 2009). Common models including the 3M 1860 additionally feature an external aluminum noseclip (*3M Technical Data Sheet: Disposable respirator, 1860, 1860S, N95*, 2018). These material differences are another source of uncertainty in the data.

In the two peer-reviewed studies on SARS-CoV-2 (Chin et al., 2020; van Doremalen et al., 2020), the environmental conditions (in terms of temperature and humidity) were similar. Stainless steel and plastic were tested in both of these papers, 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 the mean SARS-CoV-2 on stainless steel was between 4 and 7 days in (Chin et al., 2020) and ~2.3 days in (van Doremalen et al., 2020). (Chin et al., 2020) showed a 3-log decay time on plastic of 2 days and (van Doremalen et al., 2020) showed 68 hr (almost 3 days). **With**

**the limited number of studies on SARS-CoV-2 and coarse data, the variation between different studies can be *days*.**

(van Doremalen et al., 2020) 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 5 tested cases, these differences (median, extrapolated to 3-log decay) range from ~1 hr (for the short life of aerosols) to ~1 day (for cardboard).

(van Doremalen et al., 2020) gave 95% confidence intervals for each result. **These confidence intervals for the SARS-CoV-2, again extrapolating to the 3-log decay time, were approximately 1 day in all cases.**

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

In this report, we have used 3-log decay time as a standard way of comparing across different experiments. This choice is in the absence of a clear specification on the amount of viral load on an N95 FFR that constitutes “decontamination”. The initial infectious viral load will greatly impact what the infection risk remains after waiting a given period of time.

## 9. 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.

Though 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 clear qualitative conclusions that can be drawn:

- Coronaviruses survive longer at colder temperatures than at warmer temperatures, which makes storage temperatures a critical consideration in using room temperature storage for bioburden reduction.
- Coronaviruses, including SARS-CoV-2, generally live longer on surfaces that are qualitatively described as smooth or non-porous, 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 (van Doremalen et al., 2020). There is a need for a better understanding of which material properties determine virus lifetime on a surface.
- There is not a fundamental understanding of why SARS-CoV-2 might live longer one one surface than another and without that it is a challenge to extrapolate experimental results from one material to another.
- Proper donning, doffing, and hand hygiene are critical, irrespective of decontamination procedures.
- The risk of exposure to SARS-CoV-2 virus from an N95 FFR stored individually in a clean, vented, room-temperature environment goes down the longer one waits before re-using the N95 FFR.

From the reviewed literature, a 5-day waiting period encompasses the 3-log decay time for tested coronaviruses on a variety of surfaces at room temperature conditions for most of the published results, including the most recent non-peer-reviewed experiment on an N95 FFR. The exceptions are: 4–7 days for stainless steel and 7 days for the outer layer of a surgical mask tested with SARS-CoV-2 in a peer-reviewed study (Chin et al., 2020). As explained above, the experimental results on the surgical mask are conservatively judged to be relevant for this discussion of N95 FFR bioburden reduction.

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, thereby meeting the threshold for viral reduction from the FDA (FDA, 2020). The two most relevant studies show significant variation and yield **estimates between less than 1 day and about 1 week required for this bioburden reduction time for SARS-CoV-2 on materials that could be relevant for an N95 FFR. This is an area where new experimentation is urgently needed to provide more clear, actionable advice.**

Because many factors regulate viral decay, users should understand that small changes in temperature, humidity, or initial high viral loads on a N95 FFR could reduce the margin of safety. 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 and that prepare the virus in a suspension of a medium that is similar to human mucus.

Irrespective of the waiting time that is chosen, proper donning and doffing of the N95 FFR and hand hygiene are critical. Moreover, this waiting time is only an estimate and it is not expected to decontaminate the N95 FFR against other pathogens or infectious agents.



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