Heat and Humidity for Bioburden Reduction of N95 Filtering Facepiece Respirators

Keywords: decontamination, N95, respirator, heat, humidity, steam

Abstract

Introduction: The COVID-19 pandemic has caused a global shortage of single-use N95 filtering facepiece respirators (FFRs). A combination of heat and humidity is a promising method for N95 FFR decontamination in crisis-capacity conditions; however, an understanding of its effect on viral inactivation and N95 respirator function is crucial to achieving effective decontamination.

Objective: We reviewed the scientific literature on heat-based methods for decontamination of N95 FFRs contaminated with SARS-CoV-2 and viral analogues. We identified key parameters for SARS-CoV-2 bioburden reduction while preserving N95 fit and filtration, as well as methods which are likely ineffective.

Key Findings: Viral inactivation by humid heat is highly sensitive to temperature, humidity, duration of exposure, and the local microenvironment (e.g., dried saliva). A process which achieves temperatures of 70–85^{o}C and relative humidity greater than 50% for least 30 minutes is likely to inactivate SARS-CoV-2 (>3-log reduction) on N95 respirators while maintaining fit and filtration efficiency for 3–5 cycles. Dry heat is significantly less effective. Microwave-generated steam is another promising approach, although less studied, while 121^{o}C autoclave treatments may damage some N95 FFRs. Humid heat will not inactivate all microorganisms, so reprocessed N95 respirators should be reused only by the original user.

Conclusions: Effective bioburden reduction on N95 FFRs during the COVID-19 pandemic requires inactivation of SARS-CoV-2 and preservation of N95 fit and filtration. The literature suggests that humid heat protocols can achieve effective bioburden reduction. Proper industrial hygiene, biosafety controls, and clear protocols are required to reduce the risks of N95 reprocessing and reuse.

Background and Overview

The COVID-19 pandemic has led to a global shortage of single-use N95 filtering facepiece respirators (FFRs) and has forced many facilities to develop protocols for decontamination and reuse of N95 FFRs for healthcare workers. A variety of heat-based N95 decontamination methods have been proposed for the COVID-19 pandemic, including elevated temperatures alone (dry heat), elevated temperature and humidity (moist or humid heat), and the application of high-temperature steam (steam heat). These modalities differ in virucidal activity as well as their effects on N95 respirator integrity. In this review, we examine the current scientific literature on the use of heat-based methods for decontamination and bioburden reduction of N95 FFRs contaminated with SARS-CoV-2, the novel coronavirus that causes COVID-19.

Recently, moist or humid heat has been identified by the US Centers for Disease Control and Prevention (CDC) as one of the most promising methods for N95 FFR decontamination in crisis-capacity conditions. At a minimum, effective decontamination must (1) inactivate the SARS-CoV-2 virus, (2) maintain both the fit and filtration efficiency of the N95 FFR, and (3) not harm the end-user of the FFR[1]. Higher level decontamination methods such as exposure to vaporized hydrogen peroxide may also inactivate more resistant organisms, such as bacterial spores. Since data on SARS-CoV-2 inactivation on N95 FFRs are sparse, we also examined studies on other viruses, which collectively inform a set of parameters that are likely to achieve inactivation of SARS-CoV-2.

It is increasingly clear from this growing body of literature that while SARS-CoV-2 and related viruses are likely to be susceptible to heat-based inactivation, the degree of inactivation is critically sensitive to 1) temperature, 2) humidity, 3) duration of exposure, and 4) the local microenvironment (surface, mask material, and deposition solution, among others). As such, studies reporting viral inactivation should be interpreted only within the context of the experiment that was carried out. Seemingly small changes in any of these parameters (e.g., changing the deposition solution[2]) have been shown to have a large effect on viral inactivation.

While only a few studies have yet been published on heat-based inactivation of SARS-CoV-2 on N95 FFRs, these data point towards the use of temperatures greater than 70ºC for over 60 minutes to achieve sufficient viral inactivation[3]. Existing data from multiple influenza strains, bacteriophages, and a mouse coronavirus suggest that elevated humidity significantly increases heat-based inactivation of a variety of viruses on surfaces[2,4]. It has also been found in recent non-peer-reviewed reports that several N95 FFRs can withstand 5 cycles at up to 75–85ºC with 60–90% relative humidity for 30 minutes while maintaining adequate performance[5,6]. These findings together suggest that the most promising conditions for SARS-CoV-2 inactivation on N95 FFRs are likely to be **temperatures between 70–85ºC at a relative humidity greater than 50%, for 30 minutes or more.** Viral inactivation using dry heat is likely to require significantly higher temperatures and longer cycle times as compared to humid heat, though the effect of these higher temperatures on N95 performance is not well-studied[2,7]. A significant parameter space, including higher temperatures for less time, or lower temperatures at higher humidity and/or longer times, may allow for sufficient inactivation and should be investigated. Further studies with deposition solutions that more closely match saliva and/or mucus are also warranted, as heat-based viral inactivation is highly dependent on the solution used to deposit the virus on a surface[2,8–10]. As early data suggest reduced SARS-CoV-2

inactivation on metal surfaces as compared to N95 fabric[3], additional decontamination of the metal nose piece on N95 FFRs using liquid disinfectant (on the metal only) may be desired.

This review also examined the effects of repeated applications of heat and humidity on N95 FFR fit and filtration performance. Many models of N95 FFR have been shown to retain fit and filtration performance up to 3–5 cycles of humid heat treatment (see **Integrity of N95 Filtering Facepiece Respirators** and **Table 2**). The literature on N95 integrity following steam heat treatment shows mixed results depending on the protocol by which steam is applied. As these studies show, different makes and models of N95 FFR exhibit different levels of robustness under various sets of inactivation conditions. Therefore, in all cases only make- and model-appropriate inactivation protocols should be considered for implementation. Furthermore, repeated donning and doffing of an N95 is likely to reduce N95 integrity; some models failed fit tests after 5 don/doff cycles, while others maintained fit performance for over 15 don/doff cycles[11].

While this review highlights a range of conditions which are most likely to inactivate SARS-CoV-2 on an N95 while preserving respirator function, the boundary conditions for SARS-CoV-2 inactivation on N95 FFRs have not been elucidated, and further study is necessary. Furthermore, the heat and humidity conditions listed here are **not likely to inactivate other pathogens such as bacterial spores.** Therefore, treated N95 FFRs should be handled as if contaminated, and only reused by the original user in the event of an emergency shortage. This review is intended to inform healthcare professionals and decision makers in the time-critical period of the COVID-19 pandemic.

US Federal Guidelines: CDC, FDA, OSHA

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[12]. In addition, the CDC has provided guidance to hospitals on methods for decontaminating N95 FFRs during a crisis[13].

The Occupational Safety and Health Administration (OSHA) states that cosmetics or other barriers should not be present during respirator use[14]. 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[15].

After decontamination, the CDC recommends that a ‘user seal check’ is performed when the respirator is donned to ensure adequate seal[13]. 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 dons/doffs[11].

Per FDA guidelines for N95 FFR decontamination EUAs, *bioburden reduction* requires ≥3-log reduction (corresponding to a 99.9% reduction) in non-enveloped viral activity while *virucidal decontamination* requires ≥6-log reduction (corresponding to a 99.9999% reduction) in viral activity[16]. Based on this guideline, we describe a process as sufficiently “decontaminating” only when it leads to a ≥6-log reduction in viral activity and describe a 3-log reduction in viral activity as “bioburden reducing” or “reduction in viral activity”. Here, bioburden reduction and decontamination only consider virucidal activity, unless otherwise specified. Considerations of mycobactericidal or sporicidal activity have separate FDA guidelines, and are not considered

here. Heat-based N95 FFR bioburden reduction processes for SARS-CoV-2 are not expected to result in sterilization (killing of all microorganisms).

The CDC released guidance on the decontamination and reuse of N95 FFRs on March 31, 2020, which identifies the use of humid heat as one of the most promising methods for treatment of N95 respirators under crisis conditions[13]. As of June 2020, a single FDA EUA has been granted for humid heat decontamination using the STERIS STEAM Decon Cycle in AMSCO Medium Steam Sterilizers[17]. Any new methods for decontamination should be verified through an institution's internal review processes prior to implementation, which may include FDA clearance and reference to frequently updated CDC guidelines.

Mode of Action

The exact mechanism of heat- and humidity-based inactivation of SARS-CoV-2 on surfaces has not been fully elucidated. SARS-CoV-2 is an enveloped, single-stranded RNA virus[18]. Heat-based viral inactivation is thought to occur by thermal disruption of the viral capsid, viral envelope, and/or denaturation of viral proteins[19]. In droplets at room temperature, inactivation of some other enveloped viruses has been shown to be enhanced at intermediate humidity values, which is hypothesized to be due to increasing solute concentrations as droplets evaporate but are not fully dried[20,21]. Early studies also suggest that the use of heat with relative humidity over 50% will significantly increase viral inactivation on surfaces compared to heat at low humidity, though the mechanism behind this is not fully understood[2].

Importantly, heat and humidity **may not sterilize** the FFR of all pathogens, and bacterial spores, including *Clostridium difficile*, may remain[22]. This indicates that users of heat and humidity protocols for N95 decontamination or bioburden reduction must be aware of other pathogens which may survive the decontamination process. Respirators reprocessed by humid heat methods should be reused only by the original user. Proper industrial hygiene, biosafety controls, and clear reuse protocols are crucial to reduce the risks of N95 reprocessing and reuse.

Liquid Media vs. Surfaces: SARS-CoV-2 Inactivation

While there is evidence that SARS-CoV-1 and SARS-CoV-2 can be rapidly inactivated by heat when in liquid media (30 min at 56^{o}C)[23], the literature suggests that these viruses are **much more resistant to heat inactivation when on surfaces than when suspended in liquid media**. A recent, non-peer-reviewed report indicates that 70^{o}C dry heat for 30 min was NOT sufficient to reduce bioburden of SARS-CoV-2 on N95 FFR fabric[3]. Only a 1.9-log reduction was observed, as compared to the 5-log reduction observed after treatment at 56^{o}C for 30 min in liquid media. Therefore, results for heat-based viral inactivation in liquid media should not be directly compared with those for inactivation on surfaces.

Humid Heat: SARS-CoV-2 Inactivation

Studies on heat-based inactivation of SARS-CoV-2 on N95 FFRs are limited. As of August 2020, there exist only two studies of SARS-CoV-2 inactivation on N95 FFRs via the application of heat without steam (of which one is peer-reviewed)[3,24], and only one study of inactivation by steam heat[25]. In this section we review reports on the inactivation of SARS-CoV-2 and other viruses to determine a consensus set of parameters for likely inactivation of SARS-

CoV-2 without steam (see **Autoclave** and **Microwave-Generated Steam** (MGS) sections for discussion of steam protocols).

The two reports mentioned above provide the only data for heat-based inactivation of SARS-CoV-2 on an N95 FFR, though it is not currently clear that the results can be extrapolated to a real-world scenario. While both reports found sufficient inactivation (>3-log reduction) of SARS-CoV-2 after 70ºC dry heat for 60 minutes, the media used for deposition on the N95 FFRs was not listed[3,24]. If culture media was used, as is commonly done, this result may significantly *overestimate* viral inactivation from dry heat. Human saliva, mucus, and other proteins have been shown to stabilize viral particles to a greater degree than culture media (see Phi6 data in **Table 1**), indicating that a more stringent bioburden reduction protocol may be required to sufficiently inactivate SARS-CoV-2 on N95 FFRs in a hospital setting[2,8–10]. One recent non-peer-reviewed study found that deposition using DMEM overestimated viral inactivation by over 3-log as compared to deposition in human saliva, while deposition using PBS more closely matched the saliva results[2]. The SARS-CoV-2 study performed by Fischer et al., also found that 60 minutes of 70ºC dry heat only resulted in a 2-log reduction of viable virus on a stainless steel surface[3], further indicating that 70ºC dry heat may not sufficiently decontaminate N95 FFRs (which often contain metallic components). Therefore, further studies with viruses in different deposition solutions on N95 FFRs are necessary to find a safe working range of temperature, time, and humidity that will inactivate SARS-CoV-2.

Given the sparse data on SARS-CoV-2, we also analyzed the literature on humid heat inactivation of other viruses. Heat and humidity have been used to inactivate other enveloped viruses (H1N1 and H5N1 influenza) on various N95 FFRs[26,27] and surfaces[4]. N95 FFRs contaminated with some known varieties of influenza can be adequately decontaminated at temperatures over 60ºC with sufficient humidity and exposure times (see **Table 1**). One study, using a dried solution of H1N1 on stainless steel, found inactivation was more effective when either temperature or relative humidity was increased[4]. A recent non-peer-reviewed study measured inactivation of H3N2 influenza, MS2 and Phi6 bacteriophage, as well as a mouse coronavirus (murine hepatitis virus, MHV) on N95 respirators under various heat and humidity conditions[2]. This study found that for all tested humidity values >50%, all four viruses were inactivated beyond their detection limits after a 30 min treatment at 72ºC or 82ºC (>6-log for MS2 and Phi6 inoculated with PBS, >3-log for MHV and H3N2 inoculated with DMEM). However, neither MS2 nor Phi6 were sufficiently inactivated at low humidity (≤13%) after a 30 min treatment at 82ºC when deposited in saliva. This indicates that **elevated humidity is crucial for heat-based viral decontamination.** Multiple studies using various viral samples have also shown a correlation between mid to high relative humidity and increased viral inactivation[2,20,28–30]. Therefore, heat-based protocols for bioburden reduction of SARS-CoV-2 are likely to be significantly more effective at intermediate to high humidity levels and (to a lesser degree) higher temperatures.

The literature for viral inactivation on N95 FFRs indicates that relative humidity above 50% and temperatures over 70ºC for over 30 minutes can achieve >3-log decrease in active viral particles. These experiments include enveloped and non-enveloped RNA viruses, and many of them achieve viral inactivation above the detection limits of the assays performed. This includes some results showing >6-log inactivation at humidity over 50% when inoculated in representative media[2]. Given this evidence, it is likely that SARS-CoV-2 will also be sufficiently

inactivated after treatment at >50% relative humidity with temperatures of >70ºC for at least 30 minutes.

Humid Heat: N95 FFR Integrity

N95 FFRs are intended as single use respirators. There is, however, literature on the performance of N95 FFRs after multiple heat and humidity cycles, summarized in **Table 2**. This table lists, for each specific N95 FFR model, the filtration and quantitative fit tests for the most relevant studies on N95 FFR durability under heat-humidity treatments.

When considering the integrity of N95 FFRs after reprocessing, an important distinction should be made between surgical N95 FFRs (also abbreviated as SN95 FFRs) and non-surgical N95 FFRs. While both surgical and non-surgical N95 FFRs are NIOSH-certified for their filtration efficiency, surgical N95 FFRs are additionally FDA-certified for maintaining a fluid barrier. It is not well-studied whether surgical N95 FFRs maintain their fluid resistance after heat and humidity treatment. Because of the different materials used in the construction of surgical and non-surgical N95 FFRs, the integrity of these respirators under heat treatment may differ[31].

Initial studies on N95 FFR durability showed that many common N95 FFR models can undergo 1–3 cycles of 30 minutes at 60ºC and 80% relative humidity while maintaining both fit and filtration performance[32–34]. Data from more recent reports suggests that many models may be capable of withstanding multiple heat-humidity treatments at even higher temperatures up to 85ºC, and dry heat up to 95ºC. In particular, several N95 FFR models (3M 1860, 3M 1870, and 3M 8210+) have been demonstrated to pass both quantitative fit and filtration tests for at least five 30-minute cycles with temperatures of 85ºC and relative humidity of 60–85%, and with dry heat at 95ºC[5,35]. Another set of models (3M 1860S, 3M 8110S, 3M 8210S, 3M 9105S) was found to pass both fit and filtration tests after 10 60-minute cycles of 70ºC and 50% humidity[7]. Yet another set of models (3M 8200, 3M 8511, and more) were shown to pass quantitative fit tests for up to at least five 30-minute cycles of dry heat at 75ºC[36]. A single non-healthcare model (3M 9211+) was found to maintain fit after 2 cycles of 70ºC dry heat, though fit became unacceptable after 3 cycles[3]. One recent study evaluated filtration of 3M 8210 respirators at a wide range of particle sizes, and found that filtration remained above 95% for all particle sizes after 10 cycles of dry or steam heat[37]. Another recent study supports this result, and indicates that the filtration efficiency of the meltblown fabric used as a filtering material in some N95 FFRs may be unaffected for up to twenty 20-minute cycles at elevated temperature (75ºC) and humidity (100%)[38]. Four models (including 3M 8210) tested under these conditions showed no degradation in filtration efficiency after 20 cycles, though fit was not measured.

The literature suggests that N95 FFR models have varying susceptibilities to elevated temperature and humidity, so any protocol implemented should be tested with the specific N95 FFR models used locally. **See Table 2 for a list of heat, humidity, and cycle parameters that have been tested on various N95 FFR models.** For healthcare personnel utilizing any kind of FFR, a user seal check is crucial before reuse to ensure the respirator still seals properly to the face[13]. Finally, as an important additional consideration for N95 FFR reuse, repeated donning/doffing has been shown to have an impact on N95 integrity: for some N95 models, fit was found to fall below OSHA standards after 5 don/doff cycles, while others maintained fit for >15 don/doff cycles[11]. Higher temperature and humidity will likely lead to more effective inactivation of highly-resistant microorganisms, which warrants studies of N95 FFR durability at

high humidity and temperatures >85ºC for common N95 FFR models. This being said, the current literature indicates that 85ºC is the highest temperature which has been studied at high humidity and found to preserve fit and filtration for multiple models of N95 FFR. Given the requirements for SARS-CoV-2 bioburden reduction mentioned above, the range of parameters for inactivation of SARS-CoV-2 without compromising N95 respirator integrity is likely to be **temperatures of 70–85ºC and relative humidity greater than 50% for least 30 minutes.** As discussed earlier, the impact of multiple cycles of humid heat bioburden reduction on N95 performance may vary by model, so all protocols require careful validation with the N95 model and cycle parameters used.

Autoclave: SARS-CoV-2 Inactivation and N95 FFR Integrity

Autoclave treatment is a readily-accessible hospital sterilization procedure that has the potential to be employed for decontamination and reuse of N95 FFRs. While there are few studies specifically examining the inactivation of SARS-CoV-2 on these respirators under autoclave treatment, there is at least one piece of recent evidence, from a non-peer-reviewed report, suggesting that a 15 minute autoclave cycle at 121ºC can effectively inactivate the virus on N95 FFRs (see **Table 1**)[25]. Furthermore, autoclave treatment at 121ºC for 30 minutes is considered a general sterilization process in medical settings[39].

There exists a handful of studies on N95 FFR integrity after autoclave treatment, included in **Table 2**. This data shows that the impact of autoclave treatment depends on the style of the specific N95 FFR model (molded vs. pleated). Studies indicate that three molded models (3M 1860, 3M 8000, and 3M 8210) fail fit tests after only one or two cycles of autoclave treatment, while some layered fabric, pleated models (3M 1870 and 3M 1862+) maintain fit performance for up to ten cycles of autoclave treatment[25,40,41]. There is limited data on how autoclave treatment impacts filtration efficiency, but a recent study indicates that filtration performance may be significantly reduced (below the 95% NIOSH standard for N95 FFRs) after multiple autoclave cycles. In this study, the filtration efficiency of two layered fabric, pleated models (3M 1870 and 3M 8210+) decreased from ~99-100% to ~94–95% after five cycles of autoclave treatment[35]. Additional autoclave studies that include filtration tests are required to verify and supplement these findings. More generally, given the limited amount of data, additional studies are needed in order to fully understand the effects of autoclave treatment on N95 FFR durability for different models. However, in view of the demonstrated loss of filtration efficiency, as well as fit damage observed for molded N95 models, **the current data suggests 121ºC autoclave treatment may not be appropriate for N95 FFR decontamination.**

Microwave-Generated Steam: SARS-CoV-2 Inactivation and N95 FFR Integrity

While there is limited literature on the deactivation of SARS-coronaviruses via microwave-oven generated steam (MGS) treatment, studies examining the bioburden reduction of N95 FFRs containing influenza viruses (H5N1 and H1N1) or bacteriophage MS2 suggest that MGS treatment can be an effective means of decontaminating FFRs of some viruses. 2 minutes of steam treatment over a water reservoir in a 1,250 W microwave oven was found to inactivate influenza viruses by over 3.3-log, and 1.5 minutes of steam treatment in a 1,100 W microwave was found to inactivate MS2 bacteriophage by 3.1-log[26,27,42]. These studies caution that only areas of the respirator which are exposed to steam are likely to be decontaminated, so MGS

protocols should ensure that all areas of the respirator are exposed. A summary of these studies is given in **Table 1**. **Specific studies of SARS-CoV-2 are limited, so the effectiveness of MGS for bioburden reduction of SARS-CoV-2 contaminated N95 FFRs cannot currently be confirmed.** Additionally, it is important to note that MGS treatment may not fully inactivate bacterial spores, or may require additional time. It was found in one study that *Bacillus cereus* spores required at least four minutes of microwave radiation to be fully destroyed on a wet sponge[43].

The literature on the durability of N95 FFRs under MGS treatment, included in **Table 2**, suggests little to no impact on structural integrity and quantitative fit after three treatment cycles in a 1,100 W microwave, though one study found respirator damage on the inner foam nose cushion and head straps[33,34,44]. Furthermore, one peer-reviewed study found that six models of N95 (3M 8210, 3M 8000, Moldex 2200, KC PFR95-270, 3M 1870, 3M 1860, models listed in Bergman et al., 2011) maintained >95% filtration efficiency after 3 cycles of microwave generated steam from a water reservoir in a 1,100 W microwave oven[32]. However, recent tests on the meltblown fabric used as the filtering material in N95 FFRs suggest that steam treatment can have adverse effects on filtration efficiency beyond three cycles[38]. Additionally, there is insufficient data on N95 fit and filtration performance after MGS treatment in high-power, 1,250 W microwave ovens used for several viral inactivation studies described above. One study using an even higher-power microwave oven observed arcing on the metal nosepiece for certain N95 models[45]. Extending these studies to test N95 FFR filtration performance beyond three treatment cycles or in higher-power microwave ovens would be beneficial to our understanding of the effects of MGS on N95 FFR durability. Given the evidence thus far, **microwave-generated steam for 2 minutes in a 1,100 W microwave oven over a water reservoir is a promising method for inactivation of SARS-CoV-2 on an N95 respirator, though N95 filtration efficiency has not been characterized beyond 3 cycles.**

When evaluating MGS as a method of N95 bioburden reduction, it is also important to consider variations in power and geometry between different microwave models. In particular, the impact of powers higher than 1,100 W on N95 integrity is not well characterized, and merits caution. The metallic components of many N95 FFR models (e.g. nosepieces) may present additional risks due to extreme heat or sparking[45], though no such effects have been reported for 1,100 W or 1,250 W microwave ovens in studies to date[26,32–34,44]. N95 FFRs have been shown to melt in microwave ovens in the absence of steam[31], and care should be taken to introduce steam in an appropriate manner. In the literature, steam is introduced either by placing an N95 FFR above a water reservoir or by sealing it within a commercial microwave steam bag[42]. MGS treatment may be sensitive to the N95 model used and the specific protocol employed (water reservoir vs. steam bag). The references in **Tables 1 and 2** may be consulted for details on their specific implementation.

Table 1. Impact of heat & humidity on SARS-CoV-2 and other viruses on N95 FFRs and surfaces

Strain(s) (medium, if known)	Surface	Temp & RH (Method)[c]	Time (min)	Effectiveness (log reduction)	Refs.
SARS-CoV-2 (unknown)	3M 1860S, 8110S, 8210S, 9105S	70°C, dry heat	60	>3.0	A
	AO Safety N9504C (N95 fabric)		30 60	1.9 (Insufficient) >3.3	
	Stainless steel 304		60	2.0 (Insufficient)	*B
SARS-CoV-2 (Bovine serum albumin, tryptone, mucin)	3M 1860 & 1870 3M Vflex 1804 AO Safety 1054	121°C, steam (autoclave)	15	≥4.6 ≥5.3 ≥5.6	*C
Murine coronavirus MHV (DMEM)	3M 1860	72°C, 1% RH 82°C, 1% RH 72°C, 25%RH	30	1.25 (Insufficient) 2.71 (Insufficient) >3.5	*G
Influenza H1N1 (mucin, aerosol and/or droplets)	3M 1860, 3M 1870, 3M 8210, 3M 8000 KC PFR95-270 Moldex 2200	65 ± 5°C, 85% RH	30	>3.0–7.0 (FFR-dependent)	D
		(1,250 W MGS[a], water reservoir)	2	>3.3–6.3 (FFR-dependent)	
Influenza H1N1 (unknown)	Stainless steel	60°C, 25% RH 60°C, 50% RH 60°C, 75% RH 65°C, 25% RH 65°C, 50% RH	30	1.5 (Insufficient) >5.0 >5.2 2.2 (Insufficient) >5.1	E
Influenza H5N1 (aerosolized allantoic fluid)	3M 1860S 3M 1870	65°C, humid heat	30	>4.62 >4.65	F
	3M 1860S 3M 1870	(1,250 W MGS[a], water reservoir)	2	>4.81 >4.79	
Bacteriophage MS2[b] (phosphate-buffered saline)	3M 1860	72°C, 25% RH 72°C, 36% RH 72°C, 48% RH	30	1.4 (Insufficient) 3.7 >6.7	*G
Bacteriophage MS2[b] (ATCC medium 271 or unknown medium)	3M 1870 KC PFR95-270 Moldex 2200	(1,100 W MGS[a], in steam bag)	1.5	3.1 3.45 ≥3.1	H
	3M 1860	(1,100 W MGS[a], water reservoir)	3	5	I
Phi6 (DMEM) Phi6 (PBS) Phi6 (Saliva) Phi6 (Saliva) Phi6 (PBS)	3M 1860	72°C, 13%RH 72°C, 13%RH 72°C, 13%RH 82°C, 13%RH 72°C, 48%RH	30	4.3 1.62 (Insufficient) 0.95 (Insufficient) 2.62 (Insufficient) 7.09	*G
Tulane Virus[b,d]	3M 1860	100°C, 5% RH	50	>5.2	J
Rotavirus OSU[b,d]				>6.6	
Adenovirus[b,d]				>4.0	
Transmissible gastroenteritis virus[d]				>4.7	

A: (Daeschler et al., 2020)[24], B: (Fischer et al., 2020)[3], C: (Kumar et al., 2020)[25], D: (Heimbuch et al., 2011)[26], E: (McDevitt et al., 2010)[4], F: (Lore et al., 2012)[27], G: (Rockey et al., 2020)[2], H: (Fisher et al., 2011)[42], I: (Zulauf et al., 2020)[44], J: (Oh et al., 2020)[7]

* = Not peer-reviewed

a. Microwave oven generated steam. Listed power is microwave specification; actual power may be somewhat lower

b. Non-enveloped virus; may be more resistant than SARS-CoV-2 or influenza to certain treatments

c. Heating method is via oven, unless otherwise specified

d. All viruses from (Oh et al., 2020) were inoculated after mixing 1:1 with an artificial saliva solution

Table 2. Impact of heat-humidity treatment on N95 FFR fit and filtration efficiency

Model	Temp. & rel. humidity (30 min cycles)	# cycles filtration tested	# cycles fit tested	Autoclave 121°C steam, 15 min	MGS 1,100 W[d], 2 min	Refs.
3M 1860	85°C, 60–85% 70°C, 50% 100°C, dry, 50 min	Passed[a] 5 Passed 10 Passed 20	Passed[b] 5 Passed 15 Passed 20	Failed fit after 1-2 cycles	Filtration passed 3 cycles Passed fit after 20 cycles	A, *B, *C, D, E, F, G, H, I
3M 8210+	85°C, 60–85% 95°C, dry	Passed 5 Passed 5	Passed 5 Passed 5	Failed fit after 1 cycle Close to failing filtration (~95% after 5 cycles)	-	A, *B
3M 1870	85°C, 60–85% 95°C, dry	Passed 5 Passed 5	Passed 5 Passed 5	Passed fit after 10 cycles Failed filtration (<95% after 5 cycles)	Passed fit and filtration after 3 cycles	*B, *C, D, E, O
3M 8000	60°C, 80%	Passed 3	Passed 1	Fit and filtration failed after 1 cycle	Filtration passed 3 cycles Passed fit after 1 cycle	D, F, P
Moldex 2200	60°C, 80%	Passed 3	Passed 1	-	Filtration passed 3 cycles Passed fit after 1 cycle	D, O, P
KC PFR95-270	60°C, 80%	Passed 3	Passed 3[c]	-	Passed fit and filtration after 3 cycles	D, E, O
3M 8210	75°C, 90% 85°C, 100%	- Passed 20	Passed 10 -	Failed fit after 1 cycle	Filtration passed 3 cycles Passed fit after 1 cycle	*C, D, I, *J, L, P, *Q, *R
3M 8110S	70°C, 50%	Passed 10	Passed 15	-	-	I
3M 9105S	70°C, 50%	Passed 10	Passed 15	-	-	I
3M 8200	75°C, dry	-	Passed 5	-	-	*K
3M 8511	75°C, dry	-	Passed 5	-	-	*K
4C Air	75°C, dry 85°C, 100%	- Passed 20	Passed 5 -	-	-	*K L
Jackson 20	75°C, dry	-	Passed 5	-	-	*K
3M 9211+	70°C, dry	-	Failed after 3 cycles	-	-	*M
3M 9210	-	-	-	Fit passed 10 cycles	-	*C
3M 1804S	-	-	-	Fit passed 10 cycles	-	*C
3M 1862+	-	-	-	Filtration passed 5 cycles	-	N
Aearo 1054S	-	-	-	Fit passed 10 cycles	-	*C
Cardinal Health	-	-	-	-	Filtration passed 1 cycle (1.5 min)	O

A: (Anderegg et al., 2020)[5], B: (Meisenhelder et al., 2020)[35], C: (Kumar et al., 2020)[25], D: (Bergman et al., 2010)[32], E: (Bergman et al., 2011)[33], F: (Viscusi et al., 2007)[40], G: (Zulauf et al., 2020)[44], H: (Oh et al., 2020)[7], I: (Daeschler et al., 2020)[24], J: (Massey et al., 2020)[6], K: (Price et al., 2020)[36], L: (Liao et al., 2020)[38], M: (Fischer et al., 2020)[3], N: (van Straten et al., 2020)[41], O: (Fisher et al., 2011)[42], P: (Viscusi et al., 2011)[34], Q: (3M, 2020)[46], R: (Ou et al., 2020)[37]

* = Not peer-reviewed

a. “Passed” implies that filtration efficiency was >95% after the specified number of cycles.
b. “Passed” implies that quantitative fit tests resulted in fit factors > 100.
c. Fit tests were performed with 15-minute cycles, rather than 30-minute cycles used in most literature.
d. Studies cited here for MGS all used 1,100 W rated microwaves. The authors note that the actual power might have been lower.

Implementation Strategies

Many hospitals are currently equipped with or can readily procure devices that can achieve the 70–85ºC temperatures and >50% humidity mentioned above, including warming cabinets, convection ovens, circulating water baths, autoclaves, or microbial incubators. Devices with direct heating elements should not be used, as they create local temperatures that are higher than the target, therefore risking damage to the respirator. Target humidity could be achieved in heating devices, for example, by temporarily placing N95 FFRs in impermeable heat-stable boxes (e.g., plastic containers) with a source of moisture inside each box, or by isolating N95 FFRs in permeable containers and increasing the humidity of the heating device[5]. This approach may be adapted for low-resource settings by using gas-powered stoves to create a heated water bath[47]. Individual containment of N95 FFRs is recommended, as it ensures that N95 FFRs are kept physically separated (reducing possible cross contamination) and enables decontaminated N95 FFRs to be returned to their original users. We emphasize that airing of N95 FFRs *immediately* after a thermal cycle is recommended and could reduce risk of pathogen growth. Crucially, since humid heat is unlikely to inactivate all pathogens on N95 FFRs, respirators should be considered contaminated both before and after humid heat treatment. **Proper infection control workflows for respirator collection, bioburden reduction, and re-distribution to the original user are required to prevent cross contamination.**

For any given device and method, the critical process parameters should be validated to ensure proper control and performance. It is important to determine that any chosen method is able to achieve and remain at the target temperature and relative humidity for the target time, with maximal spatial homogeneity across the device. This validation should be performed under conditions as close to regular process conditions as possible with sufficient monitoring by electronic temperature and humidity sensors. Care should be used when choosing an appropriately rated sensor. This validation should be repeated periodically at a frequency determined by the facility’s established quality control (QC) practices and the party responsible for oversight and implementation of the procedure.

In donning an N95 FFR that has been through any decontamination or bioburden reduction process, the user should perform the locally recommended steps to ensure N95 FFR fit, so as to ensure that the seal is not compromised.

Primary Risks and Unknowns

Only three studies described in this report directly examined the efficacy of decontamination of N95 FFRs contaminated with SARS-CoV-2. Recent data suggests that humidity and deposition solution (mucus, saliva, culture media, aerosolized droplets, etc.) have a strong influence on viral inactivation via heat, though further study is needed for a mechanistic understanding of these observations. Future experiments validating these effects for SARS-CoV-2 are important for improving guidance on N95 decontamination and reuse for the COVID-19 pandemic. Because viral inactivation is highly dependent on temperature, humidity, and time, quality assurance measures are critical to achieving decontamination or bioburden reduction.

Process variability in heating elements or humidity sources could result in cycles with inadequate virucidal activity.

In this review we have only examined conditions that would likely result in the inactivation of SARS-CoV-2, so **the risk of other pathogens remains.** Since the current practice of many hospitals is to keep N95 FFRs at room temperature between uses, it is crucial to evaluate whether the microbial load on an N95 will increase over time during storage. In testing heat as a possible method for viral inactivation, N95 FFRs should stay physically separated from each other and should only be reused by the same clinician.

Conclusions

When possible, unused N95 FFRs and other personal protective equipment should be provided. However, this is not always feasible in crisis situations. We are sharing this review to aid in the development of real-world processes to protect clinical staff by employing equipment and supplies that may be readily available or easily obtained. This review may help guide healthcare institutions that face the need to decontaminate and reuse N95 FFRs during the COVID-19 pandemic. For heat-humidity-based bioburden reduction, we stress that (i) after each round of bioburden reduction, a user seal check should be performed, (ii) extended cycles of doffing and re-donning may affect FFR fit, and (iii) that the FFR should not be considered fully sterilized, as more resistant organisms including bacterial spores may remain even after viral inactivation.

Our review of the available literature revealed that the conditions required for inactivation by heat and humidity are pathogen-specific. Therefore, studies to determine appropriate conditions for SARS-CoV-2 inactivation on N95 FFRs are urgently needed. Preliminary inactivation data for SARS-CoV-2 on N95 FFRs, considered alongside data for other viral pathogens, **suggests that conditions of humid heat at 70°C to 85°C with >50% relative humidity for 30 minutes are likely to achieve bioburden reduction of N95 FFRs contaminated with SARS-CoV-2.** Experiments are underway to evaluate the efficacy of heat-humidity inactivation of SARS-CoV-2 on N95 FFRs.

The available literature on autoclave treatment indicates that although certain N95 FFR model types (i.e., layered, pleated models such as the 3M 1870) can maintain fit performance after several cycles of autoclave treatment, significant reduction of filtration efficiency has been demonstrated for at least two models (3M 1870 and 3M 8210+). While there is currently limited data on filtration efficiency, this suggests autoclave treatment may not be an appropriate decontamination method for SARS-CoV-2 on N95 FFRs.

The literature on microwave-generated steam suggests that bioburden reduction may be achieved for a 2 minute cycle in a 1,100 W microwave oven with a sufficiently-sized water reservoir. Several N95 FFR models have been shown to retain fit and filtration performance after 3 cycles of microwave-generated steam treatments, but the efficacy of this treatment on other respirator models is unclear. These positive preliminary results suggest that microwave-generated steam deserves further study to verify its effectiveness on more N95 models, especially due to its high accessibility in lower-resource settings.

The strategies considered here are potentially compatible with implementation in numerous clinical settings with different heating appliances (e.g. warming cabinets, water baths, autoclaves, microbial incubators, industrial convection ovens, microwave ovens), if proper

infection control workflows are in place. These strategies focus only on inactivation of the SARS-CoV-2 virus and its surrogates, and **do not serve as a means of complete N95 sterilization**. Ultimately, we hope that this review can aid hospitals in formalizing improved N95 FFR decontamination strategies for approval with regulatory agencies to better protect the health of essential healthcare workers and front-line personnel.

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