Technical Report for Heat-Humidity-Based N95 Reuse Risk Management

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 Because of this, many of the research papers cited in this document are not yet peer reviewed.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

Summary of Updates in v2.0 Report: Updated to reflect recent additions to the literature, including new studies of the impact of heat on N95 FFR integrity, inactivation of SARS-CoV-2 in liquid media, and inactivation of SARS-CoV-2 on N95 FFRs using 1) dry heat, 2) autoclaving and 3) microwave-generated steam. An important recent finding indicates that 30 minutes of dry heat at 70 °C is not sufficient to obtain a greater than one thousand-fold (≥3-log) reduction in viral activity, which is suggested by the FDA as the necessary level of inactivation for SARS-CoV-2 on N95 FFRs (*Fischer et al., 2020; FDA, 2020). Also included is a short discussion of possible validation mechanisms to ensure stability and homogeneity of heat and humidity application.

Executive Summary

A combination of heat and humidity, often referred to as moist heat, is a known method of inactivating some pathogens on surfaces and in bulk media. Recently, moist heat has been identified by the Centers for Disease Control (CDC) as one of the most promising methods for N95 filtering facepiece respirator (N95 FFR; also colloquially referred to as 'N95 mask') decontamination. In this report, we review the available literature on decontamination of N95 FFRs containing SARS-CoV-2, the novel coronavirus that causes coronavirus disease 2019 (COVID-19), as well as related viruses. Additionally, we review literature on the impact of repeated heat and humidity cycles on N95 FFR filtration and fit integrity. Based on currently available data it is clear that the effectiveness of moist heat viral inactivation is highly sensitive to 1) temperature, 2) humidity, 3) duration of exposure, and 4) the local environment (surface, mask fiber, soiling medium—e.g. mucus or saliva). These data point towards a set of promising conditions for inactivation of SARS-CoV-2 on N95 FFRs, which include temperatures between 70–85°C, humidities between 50–85%, and process times of at least 60 minutes. Temperature and humidity should be calibrated and monitored, as heating devices can be highly variable. We additionally examine N95 decontamination by autoclave and by microwave-generated steam. While these methods show some promise, more data is required to make definite conclusions for either method.

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 (FFRs). In this document, we review the use of heat and humidity to decontaminate N95 FFRs (colloquially: 'N95 masks') with the goal of increasing the useful lifetime of N95 FFRs worn by healthcare providers during

the COVID-19 pandemic. Effective decontamination 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. We examined reports on inactivation of SARS-CoV-2 in response to heat. Since few studies have extensively tested the susceptibility of coronaviruses to heat and humidity on N95 FFRs, we also examined procedures developed in anticipation of influenza pandemics. Influenza, although not closely related to SARS-CoV-2, is another respiratory virus containing a segmented single-stranded RNA genome and a lipid envelope.

It is increasingly clear from this growing body of literature that while SARS-CoV-2 and related pathogens are likely to be susceptible to heat-based inactivation, the conditions required for this inactivation are critically sensitive to 1) temperature, 2) humidity, 3) duration of exposure, and 4) the local environment (surface, mask fiber, media used under experimental conditions). Data indicate that for process times of 60 minutes and a temperature of 70°C with no added humidity SARS-CoV-2 will be inactivated by at least 3.3-log, although the local environment for the virus in that non-peer-reviewed report may not adequately resemble that found on N95 FFRs in a clinical setting (*Fischer et al., 2020). Existing data from influenza H1N1 suggests that higher humidity will better inactivate similar (enveloped) viruses (McDevitt et al., 2010). It has also been found in recent non-peer-reviewed reports that several N95 FFRs can withstand 5 cycles at up to 75–85°C with 60–90% relative humidity for 30 minutes, while maintaining adequate performance (*Anderegg et al., 2020; *Massey et al., 2020). These findings together suggest that the most promising conditions for SARS-CoV-2 inactivation on N95 FFRs are likely to be **temperatures between 70–85°C at a relative humidity greater than 50%, for 60 minutes or more**. It is accepted that higher temperatures and longer heating times lead to more effective decontamination and could be used provided they preserve N95 FFR fit and filtration integrity. A significant parameter space including **higher temperatures at lower humidity and/or shorter times**, and similarly, **lower temperatures at higher humidity and/or longer times,** may allow for sufficient inactivation and should be investigated. Further studies with media that most closely match saliva and/or mucus are warranted, especially because the presence of such substances can protect the virus from inactivation (Darnell et al., 2004; Darnell & Taylor, 2006; Rabenau et al., 2005). As studies with SARS-CoV-2 inactivation on metal surfaces at 70 °C indicate inadequate inactivation for 60 minutes in dry heat (*Fischer et al., 2020), additional decontamination of the metal nose piece on N95 FFR using liquid disinfectant (on the metal only) may be desired.

Further SARS-CoV-2 research will be required to determine the actual minimum sufficient conditions for inactivation on N95 FFRs.

Additionally, we reviewed literature on the damage caused to FFRs subjected to repeated applications of heat. As these studies show, different makes and models of N95 FFRs 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 (see summaries in **Section 4** and **Table 2**). This summary is intended to inform healthcare professionals and decision makers in this time-critical period of the SARS-CoV-2 pandemic.

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 cosmetics 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 (Battelle, 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, 2020). Based on this guideline, we describe a process as sufficiently "decontaminating" or "inactivating" only when it leads to a ≥ 3-log reduction in viral activity. **Note that our definition of decontamination in this report, unless otherwise specified, only considers virucidal activity and does *not* consider mycobactericidal or sporicidal activity**, for which the FDA has other guidelines (FDA, 2020). **N95 FFR decontamination processes for SARS-CoV-2 do not necessarily result in sterilization (killing of all microorganisms) of the N95 FFR**.

Currently, we are not aware of any FDA EUA for heat-humidity treatments of N95 FFRs. CDC released guidance on the decontamination and reuse of N95s on March 31, 2020, which identifies the use of moist heat as one of the most promising methods for treatment of N95 respirators under crisis conditions (CDC, 2020b).

Any new methods for decontamination 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. Mode of Action

In droplets, inactivation of enveloped viruses has been shown to be enhanced at intermediate humidity values, due to increasing solute concentrations as droplets shrink but are not fully dried (Lin & Marr, 2020; Vejerano & Marr, 2018). There is currently little data on methods for disinfection of SARS-CoV-2; no definitive best practice can be identified at this time.

Further work is needed to refine this protocol for implementation. Importantly, heat and humidity **may not sterilize** the FFR of all pathogens, and bacterial spores, including *Clostridium difficile*, may remain. In particular, it was found that while 15 minutes of exposure to 85°C did lead to a >4.5-log reduction of viable *C. difficile* in a liquid medium, the bacterial

spores exhibited an ability to recover during later incubation; meanwhile, exposure to 63°C for 30 minutes led to a 50% reduction in viable spores (Rodriguez-Palacios & LeJeune, 2011).

4. SARS-CoV-2 Inactivation and N95 FFR Durability

Sections 4.1 and 4.2 below give summaries and discuss the available literature on the effects of heat-humidity treatments on SARS-CoV-2 inactivation and the functional integrity of N95 FFRs. **Sections 4.3 and 4.4** are specifically dedicated to autoclave and microwave-oven generated steam (MGS) treatments.

4.1. Potential for SARS-CoV-2 Inactivation

While there is evidence that SARS-CoV-1 and SARS-CoV-2 can be inactivated by heat when in solution (30 min at >56°C, **Appendix A**), this **does not appear to translate to their sensitivity on N95 FFR surfaces**, particularly when the viral population is in mucus or saliva droplets. A recent, non-peer-reviewed report indicates that 70°C dry heat for 30 min was NOT sufficient to inactivate SARS-CoV-2 in DMEM media on N95 FFR fabric (*Fischer et al., 2020). Only a 1.9-log reduction was observed, which is below the minimum inactivation guidance of 3-log reduction set by the FDA for viruses (FDA, 2020). While 70°C dry heat for 60 minutes did sufficiently inactivate SARS-CoV-2 (>3-log reduction) on N95 FFR fabric, details of the media used for inoculation were not provided, and may not have included mucin or other proteins that have been shown to stabilize viral particles in a real-world scenario (Darnell et al., 2004; Darnell & Taylor, 2006; Rabenau et al., 2005). DMEM, which does not contain these proteins, was listed as the viral growth media. In that same study, 60 minutes of 70°C dry heat showed only a 2-log reduction of viral concentration on a stainless steel surface (*Fischer et al., 2020), further indicating that 70°C dry heat may not sufficiently decontaminate N95 FFRs (which often contain metallic components). Therefore, **further studies with viruses in different media are necessary to find a safe working range of temperature, time, and humidity that will inactivate SARS-CoV-2.** Other recent work suggests that N95 FFRs contaminated with SARS-CoV-2 can be sufficiently decontaminated via a standard autoclave cycle at 121°C for 15 minutes, though some N95 FFR models were found to fail fit tests after more than one cycle under these conditions (*Kumar et al., 2020). Autoclave treatment is further discussed in **section 4.3**, while the literature on viral inactivation on surfaces is summarized in **Table 1**, below.

Few other studies have simultaneously evaluated the effect of heat and humidity on viral inactivation and mask fit and filtration under comparable conditions, though results evaluating FFR performance alone under these conditions are discussed in **section 4.2**.

Heat and humidity have been used to inactivate other enveloped viruses (H1N1 and H5N1 influenza) on various N95 FFRs (Heimbuch et al., 2011; Lore et al., 2012) and surfaces (McDevitt et al., 2010). N95 FFRs contaminated with influenza can be adequately decontaminated at temperatures over 60°C 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 (McDevitt et al., 2010). Multiple studies using various viral samples have shown a correlation between mid to

high relative humidity and increased viral inactivation, but 100% humidity may be less effective (Casanova et al., 2010; Prussin et al., 2018; Guan et al., 2017; Lin & Marr, 2020). It is hypothesized that heat inactivation of SARS-CoV-2 will also be more efficient at intermediate to high humidity levels, though there is currently insufficient data to support this hypothesis.

4.2. Integrity of N95 Filtering Facepiece Respirators

N95 FFRs are intended as single use respirators. There is, however, literature on the performance of N95 FFRs after multiple heat decontamination 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. A more extensive version of this summary table with all the literature surveyed can be found in **Appendix B**.

Many common N95 FFR models are able to undergo 1-3 cycles of 30 minutes at 60°C and 80% relative humidity while maintaining both fit and filtration performance (Bergman et al., 2010; Bergman et al., 2011; Viscusi et al., 2011). Several models (3M 8200, 3M 8511, and more) have recently been shown to pass fit tests for at least 5 cycles of 30 minute dry heat at 75°C (*Price et al., 2020). A recent study indicates that the filtration efficiency of the fabric used as the filtering material in N95 FFRs may remain unaffected under high temperature (75°C) and humidity (up to 100%) conditions for up to 20 30-minute cycles (*Liao et al., 2020). In general, the trend in the available data suggests that many common models may be able to withstand several heat-humidity treatments at temperatures up to 85°C and high relative humidity. Data for multiple treatment cycles and higher temperatures with both filtration and fit tests is still limited, however. Only a few models (3M 1860, 3M 8210, and 3M 8210+) have been confirmed to pass quantitative fit and filtration tests after >3 cycles with temperatures >70°C and relative humidities >50% (*Anderegg et al., 2020; *Massey et al., 2020; *3M, 2020). **Different N95 FFR models have varying susceptibilities to elevated temperatures, and durability results for one model do not necessarily extend to other models. Higher temperature and humidity will likely lead to more effective inactivation of SARS-CoV-2, which warrants exploring N95 FFR durability at temperatures >70°C and humidities >50% for other common N95 FFR models. For the specific data on various N95 FFR models, see Table 2.** Any protocol implemented should be tested with the specific N95 FFR models used locally. 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 (Price & Chu, 2020). 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 (Bergman et al., 2012).

When considering the integrity of N95 FFRs after decontamination, an important distinction should be made between surgical N95 FFRs (also abbreviated as SN95 FFR) 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 their functionality as surgical masks, e.g. protecting against fluid penetration. It is not well-studied whether surgical N95 FFRs maintain their functionality as surgical masks after 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 (Viscusi et al., 2009).

4.3. SARS-CoV-2 Inactivation and N95 FFR Integrity under Autoclave Treatment

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 121°C can effectively decontaminate N95 FFRs of the virus (*Kumar et al., 2020; see **Table 1**). Furthermore, autoclave treatment at 121°C for 30 minutes is considered a general sterilization process in medical settings (CDC, 2008).

There exist a handful of studies on N95 FFR durability under autoclave treatment. The available data, included in **Table 2**, shows that the impact of autoclave treatment depends on the specific N95 FFR model. Studies indicate that three molded models, 3M 1860, and 3M 8000, and 3M 8210, fail after only one or two cycles of autoclave treatment while some layered fabric, pleated models such as the 3M 1870 and 3M 1862+ may keep their functional integrity for up to 10 cycles of autoclave treatment (Viscusi et al., 2007; *Kumar et al., 2020; van Straten et al., 2020). Additional autoclave studies that include filtration tests are required to 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.** We are aware of ongoing experimental work in this area.

4.4. SARS-CoV-2 Inactivation and N95 FFR Integrity under Microwave-Oven Generated Steam Treatment

While there is limited literature on the deactivation of SARS-coronaviruses via microwave-oven generated steam (MGS) treatment, studies examining the decontamination of N95 FFRs containing influenzas (H5N1 and H1N1) or bacteriophage MS2 suggest that MGS treatment can be an effective means of decontaminating FFRs of some viruses. A summary of these studies is given in **Table 1**. **Specific studies of SARS-CoV-2 are limited, and the effectiveness of MGS for decontamination of SARS-CoV-2 contaminated N95 FFRs cannot currently be evaluated.** 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 (Park et al., 2006).

The literature on the durability of N95 FFRs under MGS treatment, included in **Table 2**, suggests little to no impact on functional integrity in up to three decontamination cycles, albeit with some respirator damage on the inner foam nose cushion and head straps (Bergman et al., 2010; Bergman et al., 2011; Viscusi et al., 2011). The available studies are currently limited to a small number of repeated decontamination cycles, however, and 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 decontamination cycles (*Liao et al., 2020). Additionally, there is insufficient data on N95 integrity after MGS treatment in the high-power, 1250 W microwaves used for several viral inactivation studies described above. **Extending these studies to test N95 FFRs beyond three decontamination cycles would be beneficial to our understanding of the effects of MGS on N95 FFR durability.**

When evaluating MGS as a method of N95 decontamination, it is also important to consider variations in power and geometry between different microwave models. In particular, the impact of powers higher than 1250 W on mask integrity is relatively unknown, and merits caution. The metallic components of many N95 FFR models (e.g. nosepieces) may present additional risks due to extreme heat or sparking, though no such effects have been observed in studies to date (Bergman et al., 2010; Bergman et al., 201; Viscusi et al., 2011; Heimbuch et al., 2011). N95 FFRs have been shown to melt in microwave ovens in the absence of steam (Viscusi et al., 2009), and care should be taken to introduce steam in an appropriate manner. Finally, MGS treatment may be sensitive to the specific protocol employed (e.g. placed above a water reservoir vs. contained in a steam bag), and the references in **Tables 1 and 2** should be consulted for details on their specific implementation.

5. Data Summary Tables

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

Strain(s) (medium, if known)	Surface	Temp & RH (Method)	Time (min)	Effectiveness (log reduction)	Refs.
SARS-CoV-2	AO Safety N9504C (N95 fabric)	70ºC, dry heat (oven)	30 60	1.9 (Insufficient) >3.3	A
	Stainless steel 304		60	2.0 (Insufficient)	
SARS-CoV-2 (BSA[b], tryptone, mucin)	3M 1860 & 1870 3M Vflex 1804 AO Safety 1054	121°C, steam (autoclave)	15	≥4.6 ≥5.3 ≥5.6	B
Influenza H1N1 (mucin, aerosol and/or droplets)	3M 1860 3M 1870 KC PFR95-270 3M 8210 3M 8000 Moldex 2200	65 ± 5ºC, 85% RH (oven)	30	>3.0-7.0 (FFR-dependent)	C
		(1250W MGS[a], water reservoir)	2	>3.3-6.3 (FFR-dependent)	
Influenza H1N1	Stainless steel	60°C, 25% RH 60°C, 50% RH 60°C, 75% RH 65°C, 25% RH 65°C, 50% RH 65°C, 75% RH (oven)	30	1.5 (Insufficient) >5.0 >5.2 2.2 (Insufficient) >5.1 >5.1	D
Influenza H5N1 (aerosolized allantoic fluid)	3M 1860 3M 1870	65°C, moist heat (oven)	30	>4.62 >4.65	E
	3M 1860s 3M 1870	(1250 W MGS[a], water reservoir)	2	>4.81 >4.79	
Bacteriophage MS2[c] (ATCC medium 271)	3M 1870 KC PFR95-270 Moldex 2200	(1100 W MGS[a], in steam bag)	1.5	3.1 3.45 ≥3.1	F

A: (*Fischer et al., 2020), B: (*Kumar et al., 2020), C: (Heimbuch et al., 2011), D: (McDevitt et al., 2010), E: (Lore et al., 2012), F: (Fisher et al., 2011)

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

b. Bovine serum albumin

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

Table 2. Impact of heat-humidity treatment on N95 FFR durability

Model	Temp. & Rel. Humidity (oven-based, 30 min cycles)	# cycles filtration tested	# cycles fit tested	Autoclave 121°C steam, 15 min	MGS 1100 W[e], 2 min	Refs.
3M 1860	85°C, 60–85%	Passed[a] 5	Passed[b] 5	Failed after 1-2 cycles	Passed 3 cycles	A, B, C, D, E
3M 8210+	85°C, 60–85%	Passed 5	Passed 5	-	-	A
3M 1870	60°C, 80%	Passed 3	Passed 3[c]	Fit passed 10 cycles (no filtration tests)	Passed 3 cycles	B, C, D
3M 8000	60°C, 80%	Passed 3	Passed 1	Failed after 1 cycle	Passed 1 cycle	C, E, L
Moldex 2200	60°C, 80%	Passed 3	Passed 1	-	Passed 1 cycle	C, L
KC PFR95-270	60°C, 80%	Passed 3	Passed 3[c]	-	Passed 3 cycles	C, D
3M 8210	75°C, 90%	Passed 10[d]	Passed 10	Failed after 1 cycle	Passed 1 cycle	B, C, F, L, M
3M 8200	75°C, dry	-	Passed 5	-	-	G
3M 8511	75°C, dry	-	Passed 5	-	-	G
4C Air	75°C, dry	-	Passed 5	-	-	G, H
Jackson 20	75°C, dry	-	Passed 5	-	-	G
3M 9211+	70°C, dry	-	Failed after 3 cycles	-	-	I
3M 9210	-	-	-	Fit passed 10 cycles (no filtration tests)	-	B
3M 1804S	-	-	-	Fit passed 10 cycles (no filtration tests)	-	B
3M 1862+	-	-	-	Filtration passed 5 cycles (no fit tests)	-	J
Aearo 1054S	-	-	-	Fit passed 10 cycles (no filtration tests)	-	B
Cardinal Health	-	-	-	-	Filtration passed 1 cycle (1.5 min) (no fit tests)	K

A: (*Anderegg et al., 2020), B: (*Kumar et al., 2020), C: (Bergman et al., 2010), D: (Bergman et al., 2011), E: (Viscusi et al., 2007), F: (*Massey et al., 2020), G: (*Price et al., 2020), H: (*Liao et al., 2020), I: (*Fischer et al., 2020), J: (van Straten et al., 2020), K: (Fisher et al., 2011), L: (Viscusi et al., 2011), M: (*3M, 2020)

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. Filtration tests were performed at lower temperature and humidity (65°C and 50-80%); see ref. L for details.

e. Studies cited for MGS all used 1100 W rated microwaves. The authors note that the actual power might have been lower.

6. Strategies

Many hospitals are currently equipped with or can readily buy devices that can achieve the 70–85°C temperatures and >50% humidities mentioned above, including warming cabinets, circulating water baths, autoclaves, convection ovens, 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 humidities could be accomplished in these devices, for example, by temporarily placing N95 FFRs in impermeable heat-stable plastic boxes (e.g., ziploc 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. 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.

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 process, the user should perform the locally recommended steps to ensure N95 FFR fit, so as to ensure that the seal is not compromised.

7. Primary Risks and Unknowns

Only two studies described in this report, neither of which has yet completed peer review, directly examined the efficacy of decontamination of N95 FFRs contaminated with SARS-CoV-2. Data regarding the role of relative humidity in SARS-CoV-2 inactivation via heat is forthcoming, as well as data related to the effect of inoculation media (mucus, culture media, aerosolized droplets, etc.) on N95 FFR viral inactivation.

In this review we have only examined conditions that would likely result in the inactivation of SARS-CoV-2; **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 increases after incubation in moist heat relative to incubation at room temperature.

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.

Because data suggest relative humidity is an important factor in viral inactivation, quality assurance measures are critical to achieving decontamination. Process variability in heating elements or humidity sources could result in cycles with inadequate virucidal activity.

8. Conclusions

When possible, unused N95 FFRs and other personal protective equipment should be provided; however, in crisis situations this is not always feasible. 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. We hope to guide healthcare institutions that face the need to decontaminate and reuse N95 FFRs during this COVID-19 pandemic. For heat-humidity-based decontamination, we stress that (i) after each round of decontamination, 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 bacterial and mold 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 pathogens that are likely to exhibit similar stability to SARS-CoV-2 (e.g., influenza H1N1 and H5N1 on N95 FFRs), **suggests that conditions of moist heat at 70°C to 85°C with >50% relative humidity for 60 minutes might provide a good basis for further studies on decontamination 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 literature on autoclave treatment indicates that it may be an effective SARS-CoV-2 decontamination method for certain N95 FFR models (namely layered, pleated models such as the 3M 1870), while molded FFRs such as the 3M 1860 appear to fail after only 1–2 cycles. Several N95 FFR models are able to endure up to 3 cycles of MGS treatments, but the efficacy of MGS treatment in inactivating SARS-CoV-2 is unknown given the current literature.

This document will be updated as more information becomes available. 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). 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**. This document does not evaluate the efficacy of heat or humidity on inactivation of other pathogens of concern in hospital settings. Ultimately, we hope that our summary can aid hospitals in formalizing their own N95 FFR decontamination strategies for approval with the FDA to better protect the health of essential healthcare workers and front-line personnel.

9. Appendix A: Impact of Heat on SARS-Coronaviruses in Liquid Media

Though there is little data examining inactivation of SARS-CoV-2 on N95 FFRs and other surfaces, there is evidence supporting heat inactivation of viruses suspended in liquid solution. While these data are included for completeness, there is evidence to suggest that **inactivation in solution does not imply decontamination on N95 FFRs under similar conditions.** Very recent non-peer-reviewed work indicates a >6-log decrease in infectivity of SARS-CoV-2 in liquid media after 15 minutes of exposure to 92°C, while 30–60 minutes at 56–60°C is slightly less effective (*Pastorino et al, 2020). Additionally, recent data indicate that SARS-CoV-2 in liquid media can be inactivated by exposure to 70°C for 5 minutes (Chin et al., 2020). Considering that SARS-CoV-2 and SARS-CoV-1 share 79% genome identity (Lu et al., 2020) and have similar stability of infective particles in aerosol and on fomite surfaces including plastic, stainless steel, and copper (*van Doremalen et al., 2020), we also examined the temperature sensitivity of SARS-CoV-1. When held at 60–75°C for 5–30 minutes in various liquid media, SARS-CoV-1 infectivity is reduced (see **Table S1**). It is important to note that the time and temperature for viral inactivation is dependent upon the media the virus is in, including blood or mucus (Darnell et al., 2004; Darnell & Taylor, 2006; Rabenau et al., 2005). CDC guidelines state that FFRs with visible blood, mucus or other soils should not be reused (CDC, 2020a). **These results are specific to liquid media, and do not quantitatively reflect the conditions necessary for decontamination of N95 FFRs.** A summary of the effect of heat on SARS-coronaviruses in liquid media is given in **Table S1**.

Table S1. Impact of heat on SARS-coronaviruses in liquid media
Note: Heat inactivation in liquid media DOES NOT imply similar behavior on N95 FFR surfaces

Author	Media	Temperature	Time (min)	Strain(s)	Effectiveness (log reduction)
A	VTM[b]	56ºC 70°C	30 5	SARS-CoV-2	≥4.6[a] ≥3.0[a]
B	MEM + 5% FBS +/- BSA	56ºC 60ºC 92ºC	30 60 15		>5 >5 >6
C	0, 10% & 16% BSA	60°C	20	SARS-CoV-1	≥3.5
D	MEM +/- 20% FBS		30		≥5.01
C	Human plasma	65°C	20	SARS-CoV-1	≥4.25
E	DMEM		5		≥4.5
F	EM + 10% FBS	67°C	60	SARS-CoV-1	No detectable CPE[c]
E	DMEM	75°C	15	SARS-CoV-1	≥4
F	EM + 10% FBS		30		No detectable CPE[c]
G	4% FBS	58°C 68°C	30 10	SARS-CoV-1	4.9 >4.3
H	MEM + 10% FBS, 25% human serum	60°C	30	SARS-CoV-1	>4.5

A: (Chin et al., 2020), B: (*Pastorino et al., 2020), C: (Darnell & Taylor, 2006), D: (Rabenau et al., 2005), E: (Darnell et al., 2004), F: (Duan et al., 2003), G: (Pagat et al., 2007), H: (Yunoki et al., 2004)

a. Calculated by N95DECON as minimum fold change from 5.3 +/- 0.17 log TCID50 to below the 100 TCID50 limit of detection at 95% confidence.

b. Viral Transport Medium (buffered salt solution, fetal bovine serum, antibiotics, and fungicides)

c. CPE (cytopathic effect)

10. Appendix B: Extended Data Summary Table for N95 FFR Durability

Table S2. Impact of heat-humidity treatment on N95 FFR durability (extended version)

Model	Method	Temp. & Relative Humidity	Time (min)	# cycles tested	Filtration efficiency	Fit testing[a]	Notes on respirator damage	Refs.
3M 1860	*Oven* *Oven* *Oven* *Autoclave* *MGS*	85°C, 60–85% 80 to 120°C, dry 60°C, 50–80% 121°C, steam 100°C, steam (1100[e] W)	30 60 30 15 1.5–2	5 1 10 10 3	100% 99 to 98% >95% N/A 99%	Passed N/A Passed Failed Passed	 Failed after first cycle 	A N C, D, L, M B C, D, K
3M 1870	*Oven* *Oven* *Oven* *Autoclave* *MGS*	80 to 120°C, dry 60°C, 80% 60°C, 80% 121°C, steam 100°C, steam (1100[e] W)	60 15 / 30 30 15 1.5–2	1 3 / 1 3 10 3	99 to 98% N/A 99% N/A 99%	N/A Passed N/A Passed Passed	Nose foam partly delaminated for all methods 	N D / L C B C, D, K
3M 1862+	*Autoclave*	121°C, steam	15	5	97%	N/A	Blind test indicated 3-times cycled FFRs visually indistinguishable from untreated FFRs	J
3M 8000	*Oven* *Oven* *Oven* *Autoclave* *MGS*	80 to 120°C, dry 80°C, dry 60°C, 80% 121°C, steam 100°C, steam (1100[e] W)	60 60 30 15, 30 2	1 1 3 1 3	99 to 98% 99% 99% 81%, 66% 99%	N/A N/A Passed[b] N/A Passed[b]	 No visible changes to the FFR FFRs “deformed, stiff, and mottled” 	N E C, L E C, L
3M 8210	*Oven* *Oven* *Oven* *Oven* *Autoclave* *MGS*	80 to 120°C, dry 60°C, 50–80% 75°C, dry 75°C, 90% 121°C, steam 100°C, steam (1100[e] W)	60 30 30 30 15 1.5–2	1 10 10 10 10 3	100 to 99% >95% N/A N/A N/A 100%	N/A Passed[b] Passed Passed Failed Passed[b]	 Fit factor decreased from ~150 to ~125 after 1 cycle (ref. R) Failed after first cycle 	N C, D, L, M F F B C, L, K
3M 8200	*Oven*	75°C, dry	30	5	N/A	Passed		G
3M 8210+	*Oven* *Oven*	85°C, 60–85% 75°C, dry	30 30	5 5	100% N/A	Passed Passed		A G
3M 8511	*Oven*	75°C, dry	30	5	N/A	Passed		G
3M 1804S	*Autoclave*	121°C, steam	15	10	N/A	Passed		B
3M 9210	*Autoclave*	121°C, steam	15	10	N/A	Passed		B
3M 9211+	*Oven*	70°C, dry	30	3	N/A	Failed	Fit decreased factors from ~200 to ~90	I
Aearo 1054S	*Autoclave*	121°C, steam	15	10	N/A	Passed		B
Moldex 2200	*Oven* *Oven* *MGS*	80 to 120°C, dry 60°C, 80% 100°C, steam (1100[e] W)	60 3 1.5–2	1 3 3	99 to 95% 99% 98–99%	N/A Passed[b] Passed[b]	<95% filtration efficiency for 2/3 samples at 120 °C (ref. O) 	N C[d], L C[d], L, K
KC PFR95-270	*Oven* *Oven* *Oven* *MGS*	80 to 120°C, dry 60°C, 80% 60°C, 80% 100°C, steam (1100[e] W)	60 15 / 30 30 1.5–2	1 3 / 1 3 3	99 to 95% N/A 98% 96–98%	N/A Passed N/A Passed	4 of 9 samples melted at oven temperatures above 90 °C and were not tested (ref. O) 	N D / L C[d] C, D, K
Cardinal Health	*MGS*	100°C, steam (1100[e] W)	1.5	1	100%	N/A		K
4C Air	*Oven* *Oven*	75°C, dry 75°C, dry	30 30	20 5	99% N/A	N/A Passed		H G
Jackson 20	*Oven*	75°C, dry	30	5	N/A	Passed		G
N95 fabric[c]	*Oven* *Oven* *Oven* *Steam*	75°C, dry to 100% 100°C, <30% 125°C, <30% 100°C, steam	30 10 10 10	20 20 20 10	95% 95% 91% 80%	N/A N/A N/A N/A	 	H H H H

A: (*Anderegg et al., 2020), B: (*Kumar et al., 2020), C: (Bergman et al., 2010), D: (Bergman et al., 2011), E: (Viscusi et al., 2007), F: (*Massey et al., 2020), G: (*Price et al., 2020), H: (*Liao et al., 2020), I: (*Fischer et al., 2020), J: (van Straten et al., 2020), K: (Fisher et al., 2011), L: (Viscusi et al., 2011), M: (*3M, 2020), N: (Viscusi et al., 2011)

a. “Passed” implies that quantitative fit tests resulted in fit factors > 100.

b. Fit tests were performed after a single cycle, see ref. R for details.

c. This study was performed on pre-cut meltblown fabric material used as the filtration fabric in N95 FFRs.

d. This study was performed on the “small” size versions of the models Moldex 2200 and KC PFR95-270 (named Moldex 2201 and KC PFR95-174).

e. Studies cited for MGS all used 1100 W rated microwaves. The authors note that the actual power might have been lower.

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