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The Effect of Decontamination Methods on the Functionality of N95 Respirators in Particle Removal and SARS-CoV-2 Eradication

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A B S T R A C T

Background: During the early days of COVID-19 pandemic, due to the shortage of N95 respirators in hospitals and healthcare centers, the reuse of N95 respirators was posed as a crisis capacity strategy. Several studies have evaluated the efficacy of various decontamination methods on N95 respirators of well-known and approved brands. However, fundamental question is whether decontamination and reuse methods can be applied to all types of respirators.

Methods: Six types of respirators were selected from well-known and lesser-known brands which their manufacturers claimed to be N95. The selected respirators decontaminated with dry heat, ultraviolet germicidal irradiation, and ethylene oxide methods in seven consecutive cycles and their particle filtration efficiency and pressure drop were measured before and after each decontamination cycle.

Results: As the initial measurements revealed, 4 respirators (group A) showed a sharp drop in efficiency and also, negative efficiency in removing 2.5 and 4 µm particles in most of the experiments. In these respirators (group A), the maximum efficiency in removing 0.5 µm particles was 74.4 %, while the last two respirators (group B) achieved an efficiency of 98 %. Subsequent experiments following the decontamination process revealed that the non-authentic N95 respirators within group A which were not resistant to decontamination. However, the second group demonstrated a removal rate of over 95 % of particles ranging from 0.5 to 10 µm after six consecutive decontamination cycles using all three methods. The results demonstrated that ultraviolet germicidal irradiation and ethylene oxide methods could eradicate the covid-19 virus from respirators.

Conclusion: The results indicated that decontamination can be successfully applied to original N95 respirators, not low-quality respirators, even under critical conditions.

1. Introduction

 The coronavirus disease 2019 (COVID-19) which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) was first observed in Wuhan, China, and the World Health Organization (WHO) declared a pandemic on March 11, 2020 [1]. Similar to SARS and influenza, the primary mode of transmission for Covid-19 is through respiratory aerosols released by infected individuals [2]. These aerosols are divided into two groups: fine aerosols (<

5 µm) and coarse aerosols (> 5 µm). While coarse droplets settle within one hour, fine aerosols can remain in the air for hours. Particles smaller than 5 µm can penetrate the lower respiratory system, whereas larger particles only reach the upper parts of the respiratory tract [3, 4]. The United States Centers for Disease Control and Prevention (CDC) recommends that the most useful way to prevent the spread of this infectious disease is using N95 filtering facepiece respirators (FFRs) as personal protective equipment, especially for healthcare workers (HCW_S) [2]. Furthermore,

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respirators are highly recommended for the general population to prevent the virus from entering the respiratory tract. Surgical masks are commonly used by the public, while HCWs use N95-grade respirators. The National Institute of Occupational Safety and Health (NIOSH) determined the N95-grade standard for respirators (document 42 CFR part 48). N95 respirators should have a minimum filtration efficiency of % 95 for 0.3 µm particles (aerodynamic mass mean diameter) of sodium chloride aerosols. Other countries have established equivalent grades such as FFP2 (European Union), KN95 (China), DS/ DL2 (Japan), and KF94 (South Korea) [2]. N95 respirators are composed of several layers of polypropylene nonwoven fabrics, with the central layer being the meltblown layer responsible for filtering fine particles, bacteria, and viruses. This layer is 100-1000 µm in thickness and is composed of microfibers with diameters ranging from 1-10 µm. The process of meltblown production should produce a lofty nonwoven material that creates a three-dimensional network with % 90 porosity for high air permeability and filtration of fine particles, bacteria, and viruses [5, 6]. Respirators employ five main mechanismsinterception, impact, settling, electrostatic attraction, and diffusion-to capture particles. The first three mechanisms capture larger particles, while the latter two are involved in capturing fine particles and viruses smaller than 500 nm in size [7]. Electrostatic adsorption is an important mechanism in filtering fine particles since their size is smaller than the pores of respirator layers. Therefore, charging the layers of the respirator is of particular importance. Various methods, such as corona discharge, additives with different electrostatic potentials, and the dipolar nature of fibers can induce a static charge on the filters. These charged fibers, known as electrets, play a critical role in filter technology, both in respirators and in other applications [2, 7]. During the first stage of the disease outbreak, there was a great demand for respirators among the people and health centers, leading to shortages. In late March 2020, the Association for Professionals in Infection Control and Epidemiology (APIC) reported that about half of all HCWs lacked access to respirators [8]. Lack of knowledge about the lifespan of these respirators and the fear of being infected by coronavirus also caused HCWs to change their respirators more frequently, resulting in increased consumption. According to manufacturers, N95 respirators made by famous companies have a long lifespan and can be used until they are damaged or deformed. The prices of these FFRs increased in many countries, and financing to buy these respirators was another problem that some medical centers faced. Moreover, due to the lack of respirators in some countries, many manufacturers made and marketed respirators, some of which obtained government licenses. However, reports indicated that some of these respirators were fake, although their manufacturers claimed they were N95-grade [9]. To deal with these challenges, the CDC and the World Food and Drug Administration (FDA) encouraged extended use and reuse of N95 filtering facepiece respirators, as well as non-NIOSH-approved respirators, as crisis capacity strategies. They also encouraged decontamination technologies to

disinfect N95 respirators and use them for a longer time [10]. However, in 2021, the CDC retraced their earlier crisis strategy recommendation, as the shortage conditions had been eliminated, and the FDA subsequently confirmed this decision [11]. Several researchers reported various decontamination methods for respirators [12], including hydrogen peroxide vapors and plasma gas [1, 10, 13, 14], ultraviolet germicidal irradiation (UVGI) [14-17], wet steam and heat [18, 19], ethylene oxide (ETO) [12, 20, 21] and dry heat [22]. The main purpose of these decontamination methods is to eradicate or deactivate pathogens while ensuring that the respirators maintain their efficiency and can be appropriately reused. Therefore, particle filtration efficiency (PFE) and inactivation of pathogens are two important indicators for evaluating decontamination methods. Most studies on decontamination methods have focused on N95 respirator brands approved by NIOSH or other reliable organizations. Some methods resulted in no degradation in the PFE of the respirators. However, these studies characterized the respirators using only one particle size of 0.3 um. In this study, respirators produced by anonymous manufacturers were used along with respirators made by reputable companies. The effectiveness of three confirmed decontamination methods was assessed across eight particle sizes. This study aimed to investigate the relationship between the effectiveness of decontamination methods and respirator material quality and answer the question of whether any respirator, regardless of its quality, can be decontaminated.

2. Materials and Methods

2.1 Sample selection

 Six different brands of respirators labeled A to F, were selected from the central warehouse of hospitals in Zanjan Province. The manufacturers claimed that all of these respirators were N95, and respirator F was FDA-approved. The specifications of the selected respirators are shown in Table 1. From each brand, three respirators were contaminated by three decontamination methods in 6 consecutive cycles.

2.2 Tests

 PFE and pressure drop were measured before treatment, and the primary properties of respirators were determined. After each treatment cycle, the respirators were characterized. All tests were performed under standard laboratory conditions (temperature 21 ± 2 °C, relative humidity 50 % \pm 10). Totally, 1134 tests (6 respirators \times 3 different brands \times 7 cycles \times 3 treatments \times 3 test replication) of PFE and pressure drop were conducted and the average values for each three tests that were replicated were registered. Pressure drops and PFE of respirators were measured at a flow rate of 85 l/min using NaCl as aerosol according to the NIOSH-recommended methods [23]. The experiments were performed using a preparation chamber and tester built in the Department of Environmental Health

Engineering of Zanjan University of Medical Sciences. The preparation chamber was equipped with digital humidity and temperature devices. This tester was validated during a systematically designed experiment using an N95 (3M) respirator. All samples were put in a preparation chamber at a relative humidity of 85 $% \pm 5$ and a temperature of 38 $\pm 5^{\circ}$ C for 25 h before testing based on the NIOSH method [23]. The concentration of particles in the sizes of 0.5, 0.7, 1, 2.5, 4, 5, 7, and 10 µm was measured using a laser particle counter (TES 5200) in the upstream (C_0) and downstream (C) of the respirator. The PFE was calculated as follows:

 $PFE = [(C_0-C)/C_0] \times 100$

 Here, 0.3 µm particles were not measured due to high uncertainty in measuring this size. Moreover, this study aimed to compare the performance of respirators in different conditions. It is obvious that if the respirator does not have the necessary filtration efficiency at 0.5 µm, it will not be efficient at 0.3 µm.

2.3 Decontamination methods (treatment methods)

 UVGI, dry heat, and ETO treatments were chosen because these methods do not require special equipment. Furthermore, the needed devices for sterilizing the required equipment are available in hospitals and medical centers. In previous studies, these methods were recognized as effective and user-friendly.

2.3.1 UVGI treatment

 UVGI has been used by many researchers. In this study, a special cabinet for sterilizing was designed and manufactured, and using four UV-C lamps, 32 W, 254 nm, a mean dose of 1.8 $j/cm²$ was provided. Each side of the respirator was exposed to UV radiation for 7.5 min [24].

2.3.2 ETO treatment

 Low-temperature ETO gas is used to sterilize equipment in hospitals. This study used an automatic device (*AXIS* 135) located in Mousavi Hospital in Zanjan City. This sterilizer was set on a single thermal cycle (55 °C), and a 10 g vial of ETO was utilized per cycle. Respirators were first placed in poly paper bags and then, in the sterilizer. Sterilization time was 1 h, and then the samples were aerated for 15 h and placed at room temperature for 3 h. Next, respirators were tested after 72 h.

2.3.3 Dry heat treatment

 Dry heat was provided using a laboratory oven, and the samples were placed at 70 °C for 30 min.

2.4 Viral eradication test

 In this study, the potency of decontamination methods in the eradication of coronavirus 2 (SARS-COV-2) was tested. For this purpose, the outer surface of the respirators was soaked entirely with an infected person specimen obtained

from a PCR-positive patient, using a viral transport media (VTM)solution. This procedure was conducted at the Zanjan Province Corona Reference Laboratory. The respirators divided into three groups of five respirators were prepared as follows and after 24 h each group was treated by one decontamination method. The respirators were named as follows:

 S1, S2: Contaminated with infected person specimen with treatments.

 CTRL+: Contaminated with a person's PCR-positive sample without treatments (positive control).

 CTRL-1: Contaminated with a person's PCR negative (noninfected person) sample with treatments (negative control 1).

 CTRL-2: contaminated with clean VTM media with treatments (negative control 2).

 After the respirators were dried at room temperature, they were placed in separate plastic bags and decontaminated by three methods. After decontamination, the contaminated surfaces were cut into 5-mm pieces, then immersed in VTM media and placed at 1 ± 4 °C for 24 h, then shacked in a shaker incubator at 3 °C for 3 h. Thus, 2 mL of the solution, like human samples, was transferred to a Falcon tube and sent to the laboratory. A PCR test was performed by RT-PCR method, which was also approved by WHO, in the same way it is conducted for human samples in the laboratory.

Table 1. Characteristics of the studied respirators

2.5 Statistical analysis

 For statistical analysis, analysis of variance (ANOVA) using SPSS software was used to compare the effect of variables. Each respirator model was decontaminated separately, and the results of PFE and pressure drop tests were analyzed. The

results were statistically significant if *P-value* was < 0.05. Environmental scanning electron microscope images were recorded using an FEI Quanta 200 at 10 KV in order to comparing the morphology of respirators.

3. Results and Discussion

 This study performed 1134 PFE tests on respirators. Since the average of 3 replications was entered in the calculations, statistical analyses were performed using 378 data.

3.1 Primary characterization results

 Figure 1 shows the results of PFE tests on the respirators before decontamination.

Figure 1. filtration efficiency of Respirators before decontamination cycle (0)

 As can be seen, the tested respirators are divided into two groups based on the results. The first group consists of respirators A, B, C, and D claimed to be N95, but their mean PFE values for the removal of 0.5 um particles were 71.1 %. 74.7 %, 73.2 %, 60.9 % for A, B, C and D respirators, respectively. In the second group (respirators E and F), these values were 98.3 % and 99.2 %, respectively. The PFE range in the first group of respirators was between 60 % (for respirator D), and 76 % (for respirator B), but in the second group, it was between 98.3 % and 99.2 %. The results confirmed that the respirators of group 1 could not definitely remove 95 % of the 0.3 µm particles (according to NIOSH criteria) and therefore, certainly could not be N95 grade. Additionally, a significant observation was made regarding the decayed filtration efficiency of 2.5 µm particles in group A respirators. This decay and negative efficiency were consistently observed in most of the experiments on group A respirators, both before and after the treatments, whereas group B respirators did now show this issue. The initial

pressure drop of the respirators before applying the treatments were 189 ± 2 , 163 ± 2 , 146 ± 2 , 206 ± 2 , 180 ± 2 , and 188 ± 2 Pa. for A to F respirators, respectively. The pressure drop increased during all six cycles after all treatments, but the rate of increase in respirator D was significantly different from that of other respirators. The total increase in pressure drop in respirator D in all treatments after six cycles of decontamination was 233 pa. However, in other respirators, it was between 41 and 68 pa. Except for respirator D, the pressure drop in all cases for two groups was less than the NIOSH-recommended value. Other respirators had a similar response to pressure drop after treatments, but their reaction to PFE tests was quite different**.**

3.2 Effect of treatments

 The study analyzed the effects of particle size, number of cycles, and decontamination variables on PFE values using ANOVA, which indicated their significant impact as well as the simultaneous effects of variables were significant (Table 2). Figures 2 and 3 show the PFE results of respirators A and F as samples of groups A and B, respectively, in different decontamination methods and cycles. The respirators of group A showed different responses to decontamination methods, while group B's efficiency remained unaffected by the treatments. After ETO, dry heat, and UVGT treatments, group A respirators achieved mean PFE values of 66.5 %, 58.4 %, and 59.5 %, while group B respirators maintained PFE values above 97 %. The mean PFE of 2.5 to 4 µm particles in group A respirators showed a sharp degradation and even negative efficiency after all treatments. In contrast, group B respirators demonstrated minimal degradation in PFE during the six decontamination cycles. Since group A respirators did not meet the necessary PFE requirements, virus removal and eradication experiments were conducted only on group B respirators. Respirator E, which exhibited the highest efficiency in group B, was selected for these experiments. By increasing the number of cycles, the PFE was not stable in group A. Moreover, a significant decay in PFE was observed with increasing the number of decontamination cycles (from 78 % to 48 %) regardless of particle size after all treatment methods. In group A, the efficiency reduction slope was very steeped in all respirators, and during the six cycles, a 50 % reduction in efficiency was observed. In contrast, in group B, degradation of PFE was minimal during the six cycles.

3.3 Viral eradication

Since the main purpose of using respirators is to prevent the entry of microbial particles into the human respiratory tract and respirators of group A did not have the necessary particle filtration efficiency, virus removal, and eradication experiments were performed only on group B respirators. According to the specifications and similar results of the two respirators in group B and considering safety precautions, only respirator E was selected, which showed to have the highest efficiency. Therefore, 45 respirators of F type were selected for each decontamination method.

Figure 2. Filtration efficiency of Respirator A

Table 2. Summary of ANOVA results

 Tables 3 and 4 illustrate PCR test results related to the infected and non-infected individuals, whose samples were used to contaminate the tested respirators. In Tables 3 and 4, Cycle Threshold (CT) values less than 40 are considered positive for RdRp and N genes. This study used the COVID-19 molecular detection kit made by Pishtaz Teb Zaman Company. The Taqman real-time PCR method was used for virus detection. The probe-primer mixture of this kit was designed by a dual-target gene method that simultaneously targets the protected genomic sequences of the RdRp (RNAdependent RNA polymerase) region and the N (N-protein nucleocapsid). This kit includes an internal control probe and primer RNase P (Ribonuclease P) that increase the accuracy of the sampling and extraction process to avoid falsenegative results. In all methods in positive control samples, the results were positive for both N and RDRP and negative in all negative control samples. The results were negative for both main samples of UV and ETO treatments. The respirator specifications and the layers from which the respirators are made are shown in Table 1. In order to study the structure of the respirators, 10 respirators of each brand were selected randomly, and samples were prepared from the meltblown layers, which was the main layer in particle separation and filtration. The weight per unit area of the meltblown layer was measured (Table 1), indicating that the respirators in group B had a higher weight per unit area compared to those in group A. It revealed that meltblown layer in group B respirators had greater density and assurance. Although all the respirators were made of five layers, the quality of the meltblown layers was completely different among the studied respirators. Scanning electron microscope (SEM) imaging (Figure 4) was performed to further examine the meltblown layers. The SEM images showed that the respirators in group A exhibited non-uniform texture and

had empty spaces between fibers. The only uniform respirator was from group B, which was also more efficient. While the respirators in group B had a high and quite obvious uniformity compared to those in group A, the images revealed that the diameter of the fibers of the meltblown layers was not significantly different among the respirators. Therefore, the difference in efficiency cannot be attributed to the fiber diameter. The negative efficiencies in the respirators of group A show the release of particles from the respiratory surface, which can be associated with the low quality of the layers of these respirators, especially the meltblown and spun-bond layers, and their intolerance either to air flow rate or the preparation condition (temperature and humidity). The fabrication and preparation conditions of the meltblown layer directly impact the quality of this fabric. In these respirators, the particles are released at a flow rate of 85 liters per minute, which is a high rate of human air consumption during activity. Furthermore, 2.5 µm particles can enter the deep parts of the respiratory system and leave harmful effects. Given that no significant changes were seen in the pressure drops in the respirators, one of the reasons for the low efficiency and release of particles from the surface of respirators could be the lack or poor electrostatic discharging of these respirators [2]. The loss of charges or low electrostatic charge causes the release and resuspension of particles from the filter surface. Therefore, based on the results and characteristics of the layers, the low density and presence of empty spaces on the meltblown layer and lack or poor static charge in the layers of the respirators can be considered the main reasons for the decreased efficiency in successive cycles. As the Figures indicate, PFE decreased in all respirators after the decontamination operation, although the decrease was small in group B.

Figure 3. Filtration efficiency of Respirator F

 (cc)

 Comparisons with other studies revealed that decontamination methods could be accepted with certainty, and their effects varied on different respirators. In this study, the dry heat method did not significantly affect the original respirators in group B, but a clear reduction in PFE was observed in group A respirators. The dry heat removal method also showed no visible effect, although studies have reported different results concerning the dry heat method. Yan *et al.* (2020), reported that after 10 cycles of dry heat decontamination (30 min, 10 cycles, and 77 °C), the efficiency of the studied respirators decreased [22]. In another study, Fisher *et al.* (2020) evaluated the efficiency of the dry heat method on N95 respirators. They indicated that at 60 °C for 60 min, the active SARS COV-2 had a log reduction of 4-5, and the average fit factor was < 100 after 1 and 2 cycles, but after 3 cycles, it fell below 100 [14]. Liao *et al*. (2020) decontaminated polypropylene metal sheets in 20 cycles at 75 °C for 30 min. The test was performed like the test done in our study (85 lpm, NaCl aerosol), and PFE remained above 95 % after 20 tests, while in the present study, the reduction of PFE in group A respirators was quite obvious [2]. ETO is widely used in sterilizing health, medical, laboratory, and diagnostic equipment and museum products [25]. Several studies have shown that ETO does not have a detrimental effect on particle removal efficiency, pressure drop, and physical properties of respirators. While this study found that this method significantly reduced the efficiency of poor-performing respirators made of low-quality materials, the presence of ETO residue in respirators is a major concern, and its usage as a decontamination method is not recommended by NIOSH and CDC [12, 26].

Table 3. PCR specifications of the samples taken from the COVID-19-infected and uninfected person

		Cycle Threshold	
	RdRp		RNase P
Covid-19 infected person	21	つつ	25
Covid-19 uninfected person	-		つろ

 The UVGI method, commonly used in water, wastewater, and air treatment displayed favorable results at a wavelength of 245 nm. The effectiveness of this method depends on the intensity and lifespan of the lamp, as well as the distance between the object and the lamp. The phenomenon of shadowing and soiling agents (compounds that cover microbes) can also affect the efficiency of UGVI disinfection. Numerous studies have shown the effectiveness of this method in eradicating viruses. Bergman *et al.* (2010) reported no observable effects on respirator performance and fitting parts in three consecutive cycles using this method. However, 3M testing on 1860 and 1870 models revealed that the strap lost elasticity on the 1870 model, and the nasal foam was compressed on the 1860 model after exposure to 245 nm UV light for 30 minutes [12, 24, 27]. Despite these effects on specific respirator models, the UVGI method proved highly effective in decontaminating

respirators, as it did not significantly reduce efficiency or increase pressure drop. It also demonstrated strong virus eradication capabilities. However, a limitation of this method is the requirement for cabinets with large surface areas to expose the respirators to UV radiation. PCR test results showed that both major samples were positive in the dry heat decontaminated samples. Since PCR can detect live and inactive viruses simultaneously, it can be concluded that the ETO method can eradicate the virus with higher confidence. In the UV method, the results were satisfactory, although RNase P was detected in one sample. An increase in CT compared to the original samples of the infected person indicates that the infection load is reduced, and the presence of the gene does not necessarily mean that the virus survives. The decontamination method may affect the other components of the virus and kill it. Furthermore, since ETO and UV influence the genomes of bacteria and viruses, the results confirm that these two methods are effective in eradicating the virus. The general results showed that ETO was a more effective method for decontamination in terms of the effect on the respiratory tissue and eradication of the virus, but the problem that limited its use is the residual ETO in the respirator, which should be investigated to evaluate the effect of long aeration on this ETO residue. The material of respirator layers also plays a significant role in the efficiency of decontamination methods. If the N95 respirators are not real and original, their efficiency in successive decontamination cycles significantly decreases. It is crucial for hospitals and healthcare centers to ensure authenticity of masks and respirators, with adequate supervision provided by relevant organizations. Moreover, respirator factories and workshops are required to be inspected by relevant departments. Due to the contradictory results observed in this study, implementing decontamination operations on all available respirators is unreliable and is not recommended. As a result, the CDC and NIOSH's decision to ban these methods under normal conditions seems justified.

Table 4. PCR test results of infected and control groups of respirators

Figure 4. The Scanning electron microscope (SEM) images of the meltblown fabrics of studied respirators

4. Conclusion

 Contrary to many other studies that confirmed the decontamination of respirators, the present study found that the decontamination methods in respirators of group A significantly reduced their efficiency. However, the reduction in efficiency observed after the decontamination of two respirators in group B was not significant. The effects of different decontamination methods on respirators were found to be dependent on their raw material and model. ETO and UVGI methods showed more reliable results in eradicating the COVID-19 virus. It is important to note that different results may be obtained when applying decontamination methods to other respirators from various brands. Moreover, the results indicated that the type and material of respirators play a vital role in determining the

efficiency of decontamination methods and cannot be definitively recommended as a general rule for decontamination of all respirators. Since the study only focused on COVID-19 virus, further studies are needed to determine whether decontamination methods can eradicate other pathogens, even in genuine respirators. In conclusion, it can be said that the decontamination methods should not be applied to any respirator, regardless of their constituents. Therefore, the decision made by the CDC and NIOSH to revoke the Emergency Use Authorizations for Certain Respirators and Decontamination Systems appears to be reasonable measure.

Authors' Contributions

 Zohre Farahmandkia: Data collection and organization; primary manuscript author. Leila Ghorbani: Performing laboratory tests. Hessam Mirshahabi: Virologist Performing PCR tests. Mohammad Reza Mehrasebi: Principal Investigator; manuscript author and editor.

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Conflicts of Interest

 The authors declare that they have no known competing financial interests.

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Ethical considerations

 This research project was reviewed and approved by research and technology vice chancellor of Zanjan University of Medical Sciences with ethical code: (IR.ZUMS.REC.1399.307).

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