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Original Article

A Prototype N95 Sterilizer: An Alternative Solution during Personal Protective Equipment Crisis

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ABSTRACT

The high demand for N95 masks, especially during the COVID (Coronavirus disease)-19 pandemic, has caused shortages worldwide. This study aimed to examine the sterilization ability of the portable sterilizer prototype for N95 masks and its effect on the filtration ability and changes in air resistance on the N95 mask in order to thrift personal protective equipment (PPE) use during a shortage. The sample used was an N95 mask type 1860. The mask was contaminated with 0.6-0.8 MFU Staphylococcus aureus and Escherichia coli. The sterilization methods used were Ultraviolet Germicidal Irradiation (UVGI), Heat at 75°C, and a combination of both from 1 to 120 minutes. Next, the masks were cultured in a nutrient agar medium. For aerosol penetration and air resistance tests, masks were tested before and after the sterilization process, lasting from 5 to 60 minutes. This prototype sterilizer with Heat effectively killed E. coli and S. aureus starting from 3 minutes. The filtration ability of the N95 mask was maintained at >95% even after the sterilization process with 75°C heat, UVC, or a combination of both for up to 60 minutes. There was also no significant difference in air resistance between new masks and masks that had been sterilized using a portable sterilizer. This prototype sterilizer with Heat at 75°C can effectively sterilize against both gram-positive and negative bacteria in the N95 mask without reducing the aerosol filtration ability and changing the air resistance of the N95 mask.

Keywords: *aerosol; filtration; N95; Personal Protective Equipment; sterilization*

ABSTRAK

Tingginya permintaan masker N95 terutama di masa pandemi COVID (Coronavirus disease)-19 menyebabkan kelangkaan masker di seluruh dunia. Penelitian ini bertujuan untuk menguji kemampuan sterilisasi dari prototipe portable sterilizer masker N95 dan pengaruhnya terhadap kemampuan filtrasi dan perubahan hambatan udara pada masker N95 dalam rangka penghematan penggunaan alat pelindung diri (APD) pada saat terjadi kelangkaan. Sampel yang digunakan adalah masker N95 tipe 1860. Masker dikontaminasikan dengan 0,6-0,8 MFU (McFarland unit) Staphylococcus aureus dan Escherichia coli. Metode sterilisasi yang digunakan adalah Ultraviolet Germicidal Irradiation (UVGI), panas pada suhu 75°C, dan kombinasi keduanya dalam durasi 1 hingga 120 menit. Selanjutnya, masker dikultur dalam media nutrien agar. Untuk uji penetrasi aerosol dan hambatan udara, masker akan diuji sebelum dan sesudah proses sterilisasi dengan durasi 5 hingga 60 menit. Prototipe sterilizer dengan panas 75 °C ini efektif membunuh E. coli dan S. aureus mulai dari 3 menit waktu sterilisasi. Kemampuan filtrasi

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masker N95 tetap terjaga >95% meskipun telah melalui proses sterilisasi dengan panas 75°C, UVC, atau kombinasi keduanya hingga 60 menit. Selain itu, tidak ada perbedaan yang signifikan dalam hambatan udara antara masker baru dan masker yang telah disterilkan menggunakan alat sterilisasi portabel. Prototipe alat sterilisasi dengan panas pada suhu 75°C ini dapat secara efektif mensterilkan bakteri gram positif dan negatif pada masker N95 tanpa mengurangi kemampuan filtrasi aerosol dan mengubah hambatan udara masker N95.

Kata kunci: *aerosol; Alat Pelindung Diri; filtrasi; N95; sterilisasi*

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INTRODUCTION

Infectious diseases are one of the leading causes of death in the world. The World Health Organization (WHO) reports that lower respiratory tract infections are the fourth leading cause of death globally and the second most common cause of death in developing countries.[1] The easy transmission of disease from animals to humans or fellow humans makes infectious diseases have a reasonably high incidence. One method of transmission that transmits very quickly is through aerosol or airborne. This transmission occurs when an infected person expels droplets or aerosols when talking, singing, coughing, or sneezing.[2,3] One way to prevent this disease's transmission is using face masks. The use of face masks can reduce a person's chance of being infected by up to 90%.[4] One type of face mask is recommended for health workers as personal protective equipment (PPE) on duty is the N95 mask. The high demand for N95 masks, especially during the COVID-19 pandemic, has caused shortages worldwide.[5]

The Center for Disease Control and Prevention (CDC) publishes guidelines for reusing N95 masks during the PPE crisis. This reuse must pay attention to several things regarding N95 masks. Some things that need to be considered in mask reuse are contamination and filtration performance. In addition, it is also necessary to pay attention to the mask damage and its fitting.[6] Many studies have been conducted on the sterilization methods of N95 masks for reuse. Some methods studied were evaporation, dry heat, Ultraviolet C (UVC), gamma radiation, hydrogen peroxide, boiling in water, and liquid disinfectants such as chlorine and alcohol.[7–9] Some of these methods damaged the mask, but UVC and dry heat were reported to maintain a safe mask's filtration performance.[7]

Based on this problem, we designed a special portable sterilizer for N95 masks, which can eliminate pathogens but does not affect mask performance. This sterilizer is compact, easy to use, and can be used anywhere.

MATERIALS AND METHODS

Materials & Tools

The masks used were the N95 type 1860 3M masks. *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 isolates were used to contaminate the masks. Particle counter HTI type HT9600, Manometer HT-1890, and Nebulizer OMICRON MY-520A were used as aerosol generators. The particle counter could measure aerosol particles starting from 0.3 µm, 2.5 µm, to 10 µm. This device also had a maximum measurement capability of 10^7 piece/L particles and resolution up to 1 piece/L.

Methods

Prototype Design of Portable Sterilizer

This portable sterilizer was made using a 15 mm Medium Density Fiberboard (MDF). Dimensions were about 37 cm high, 21.8 cm wide, and 21 cm depth. Inside the sterilization chamber, a wire mesh was placed as a base, and hangers were attached on the sides so that the masks could be hung vertically on the side

walls so that all masks could get an even heat between one another. The prototype of the portable sterilizer is shown in Figures 1A and 1B.

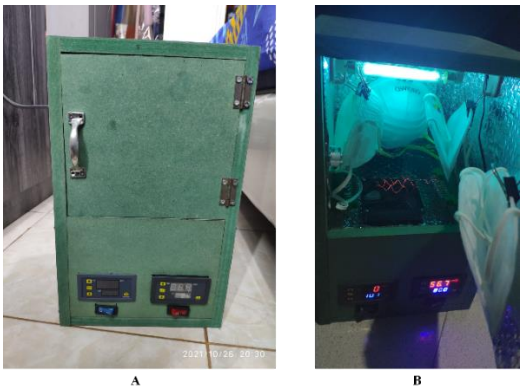

Figure 1. Portable sterilizer (A) Front view, (B) Inside view of portable sterilizer prototype when operating

This device used the heat method for sterilization resulting from converting electrical energy into heat. The heating element used was Nichrome Ni80 wire (80% nickel, 20% chromium). A 4W UVC lamp was also added to maximize the sterilization effectiveness.

The power source of this tool used a 240 W power supply with a 12 V DC and 20 A current. This tool could produce 200 W of thermal energy to heat the sterilization chamber by radiation. A 9 cm fan would help circulate the hot air produced by the heating element, so the heat was more evenly distributed throughout the sterilizer chamber. The thermostat was used to control the heat. It was set on at 75°C and off at 75.5°C. A timer was used to adjust the duration of sterilization according to the treatment group.

Mask Filtration Efficiency Tester Design

The mask filtration efficiency tester was designed as shown in Figures 2A and 2B. This tool was tried to imitate the working principle of the standard tool for measuring mask filtration capability, TSI Automated Filter Tester 8130A. This tool was made from polyvinyl chloride (PVC) tube with an inside diameter of about 6.35 cm (2.5 Inches) and a length of about 65 cm.

The aerosol produced by the nebulizer would be mixed with room air using a blower which would then be blown into the intake of the filtration tester tool. The nebulizer will automatically generate a variable-sized particle. These particles' sizes will be distinguished using particle counter and calculated in numbers. The vacuum fan would suck the air that had been mixed with the NaCl aerosol. The mask would be placed in the middle of the tool to filter out aerosol particles that had been sucked. Particle counters were placed in space before filtration occurred or in front of the mask (Upstream) and space after the air was filtered or behind the mask (Downstream). In addition, Manometer sensors were also placed in the two chambers to measure the air pressure difference between chambers.

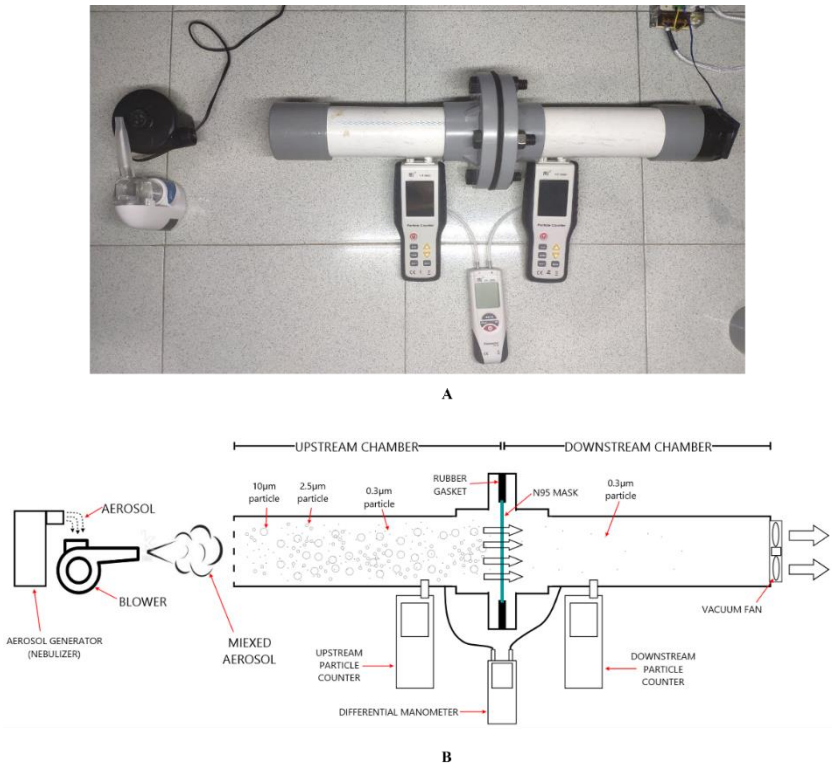

Figure 2. The mask filtration efficiency tester (A) Overall view, (B) The working principle of the mask filtration efficiency tester

Sterilization Test

The experiment was conducted in Microbiology Laboratory, Hasanuddin University Hospital, Makassar, Indonesia. The masks were artificially contaminated by the method used by Ibáñez et al. [10] with modifications. Airborne pathogens could not be used in this study due to limited laboratory biosafety availability. In this investigation, *E. coli* and *S. aureus* microorganisms were employed instead. The mask was cut into small pieces about 20 x 7.5 mm so that it would later fit into the microcentrifuge tube during the elution process. All mask samples were clamped using a wooden clamp, put in a clear plastic bag, and pre-sterilized at 90°C for 60 minutes to eliminate environmental contamination. The mask was removed from the plastic and contaminated with 100 µl of a 0.6-0.8 MFU (McFarland Unit) solution of *S. aureus* or *E. coli*.

After that, the mask was put back into a plastic bag and the sterilization process was carried out using (1) UVC, (2) 75°C heat (temperature to inactivate *S. aureus* and *E. coli*),[11,12] and (3) a combination of both in a duration of 1, 3, 5, 10, 30, 60, 90, and up to 120 minutes. For the control, we used an uncontaminated mask as a negative control and an unsterilized mask as a positive control.

After the sterilization process was complete, the mask was drowned in 0.5 mL of saline solution in a microcentrifuge tube and vortexed to elute the bacteria contained in the mask. The saline solution was then dropped as much as 0.1 mL onto a nutrient agar medium and spread. The medium was incubated at 37°C for 24 hours. After 24 hours, bacterial growth would be observed. Culture results showing more than 30 colonies were categorized as positive, while less than 30 were categorized as negative because they were too few to represent the sample. Cultures that produced more than 300 bacterial colonies were considered too many to count (TMTC).[13]

Aerosol Penetration and Air Resistant Test

In preparation, the stiff edges of the mask were cut to remove the rigid structure of the mask. This was intended to make it easier for the mask to be inserted into the filtration test device later. After that, the mask was tested for its filtration ability (F) by calculating the difference in the number of 0.3 µm sized particles between the Upstream (Us) and Downstream (Ds) spaces using the formula:

$$F = \frac{(Us - Ds)}{Us} x100$$

Air resistance was tested by calculating the difference in air pressure in the Us and Ds chambers. After obtaining the initial data, the mask was sterilized using UVC, 75°C heat, or a combination of both for 5, 10, 30, and 60 minutes, respectively. After the sterilization process, the mask was tested again for its sterilization ability and air resistance as in the previous method. The results were about 20 x 7.5 mm compared before and after the sterilization process. The air pressure difference data were entered into Microsoft Excel software and tested using a paired T-test to find their significance. This method was a modification of the method used by Gobi et al.[14] and Vossen et al.[15] to determine the mask's filtration efficiency and air permeability. Briefly, the research flow is depicted in Figure 3.

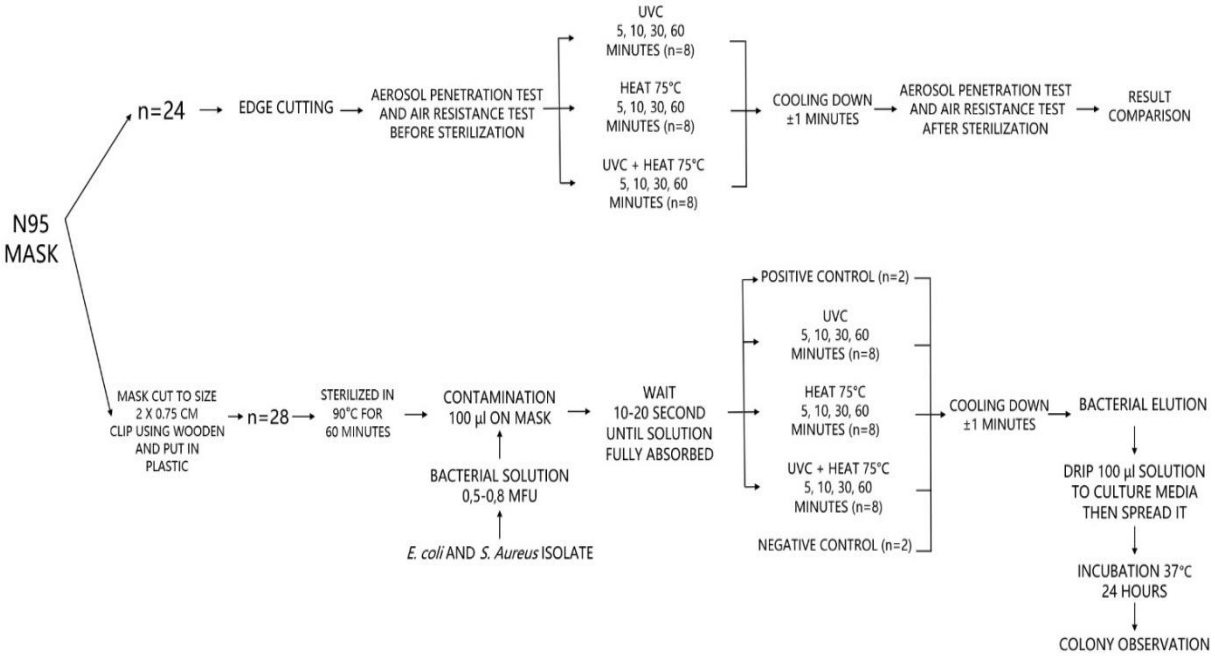

Figure 3. Research Flow

RESULTS AND DISCUSSION

Sterilization Test

Most of the culture results made more than 300 colonies of bacteria and were considered Too Many To Count (TMTC). More than 30 colonies were categorized as positive results, while less than 30 were categorized as negative because they were too few to represent the sample. Some plates also showed the results of colonies stacking up on each other due to the uneven distribution of eluted solution during preparation.

The results of mask culture after sterilization using the N95 prototype sterilizer are summarized in Table I. Using a portable sterilizer with the heat of 75°C gave negative culture results for both gram-positive and gram-negative bacteria from 5 minutes to 60 minutes of sterilization duration, as shown in Figure 4.

Table 1. Mask Culture Results after Sterilization using the N95 Prototype Sterilizer

Sterilization Method	Culture Result	
	***E. coli* (0.81 MFU)**	***S. aureus* (0.7 MFU)**
Positive Control	**Positive**	**Positive**
Negative Control	Negative	Negative
UVC 5 minutes	**Positive**	**Positive**
UVC 10 minutes	**Positive**	**Positive**
UVC 30 minutes	**Positive**	**Positive**
UVC 60 minutes	**Positive**	**Positive**
Heat 75°C 5 minutes	Negative	Negative
Heat 75°C 10 minutes	Negative	Negative
Heat 75°C 30 minutes	Negative	Negative
Heat 75°C 60 minutes	Negative	Negative
UVC + Heat 75°C 5 minutes	Negative	Negative
UVC + Heat 75°C 10 minutes	Negative	Negative
UVC + Heat 75°C 30 minutes	Negative	Negative
UVC + Heat 75°C 60 minutes	Negative	Negative

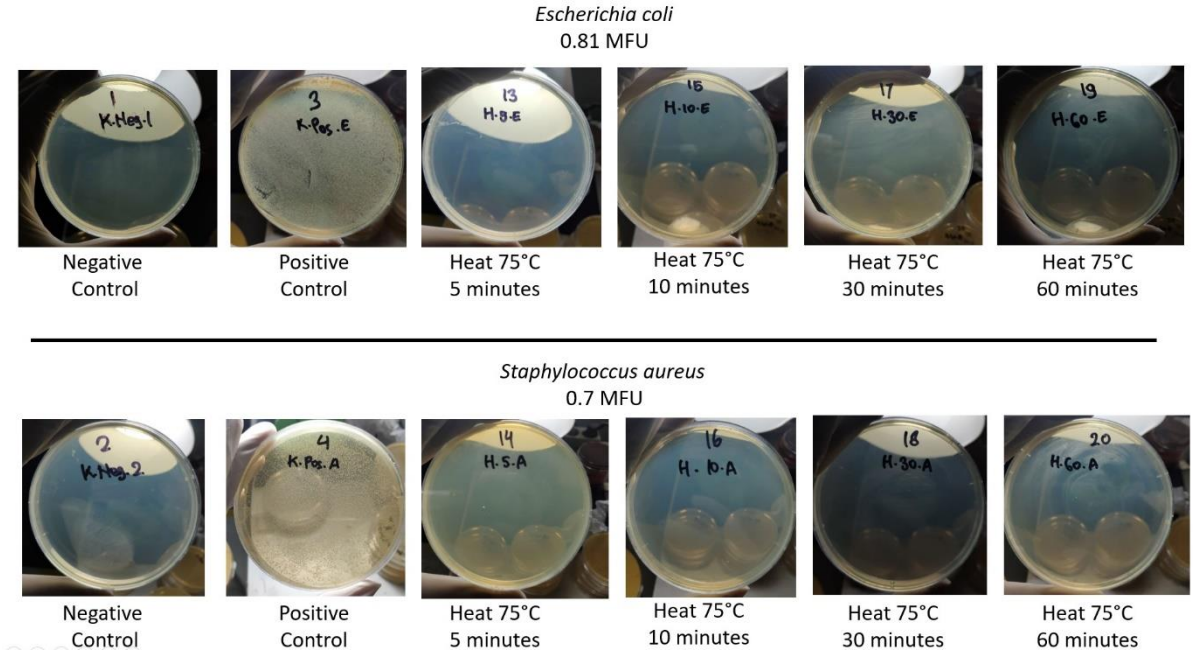

Figure 4. Mask Culture Results after Sterilization using heat of 75°C

We tried to reduce the duration of heat exposure to 1 and 3 minutes, respectively, to see the lower limit of this portable sterilizer's performance. At 1 minute, there was still colony growth, especially in *E. coli* culture, while in the 3 minutes group, the two groups of bacteria did not show any colony growth on the medium.

In contrast, sterilization using UVC gave the opposite result. This portable sterilizer could not eradicate *S. aureus* and *E. coli* even with 60 minutes of sterilization. The documentation of the mask culture results after sterilization using the prototype N95 with the UVC method is shown in Figure 5.

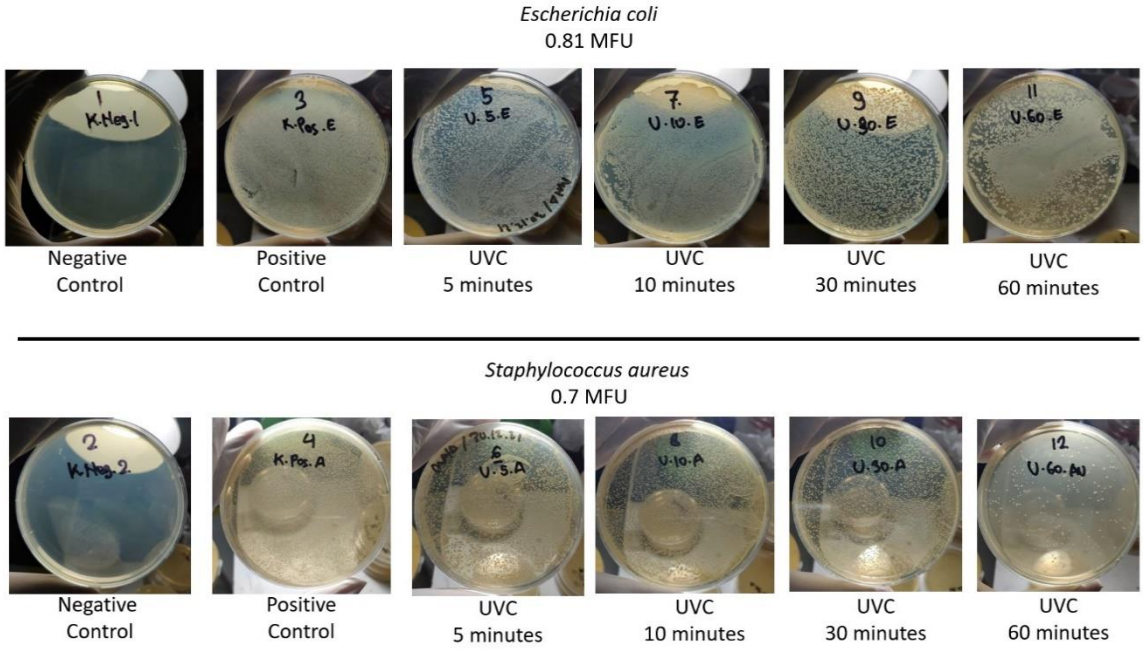

Figure 5. Mask Culture Results after Sterilization using UVC

Therefore, we added the duration of the mask's exposure to UVC rays to 90 to 120 minutes, but that still was not able to give negative culture from both bacteria, as shown in Table 2. Culture result documentation can be shown in supplementary Figure 6.

Using a combination of heat at 75°C and UVC gave no different culture results than using heat alone. Culture documentation is shown in Figure 7. Culture testing was done in a duplex to get more accurate results. There was no difference in culture results between the first and second experiments.

Table 2. Mask Culture Results in Shortened and Extended Sterilization Durations

Sterilization Method	Culture Result	
	***E. coli* (0.64 MFU)**	***S. aureus* (0.82 MFU)**
Positive Control	**Positive**	**Positive**
Negative Control	Negative	Negative
Heat 75°C for 1 minute	**Positive**	Negative[1]
Heat 75°C for 3 minutes	Negative	Negative
UVC + Heat 75°C 1 minute	**Positive**	Negative
UVC + Heat 75°C for 3 minutes	Negative	Negative
UVC 90 Minutes	**Positive**	**Positive**
UVC 120 Minutes	**Positive**	**Positive**

[1]only a colony was found

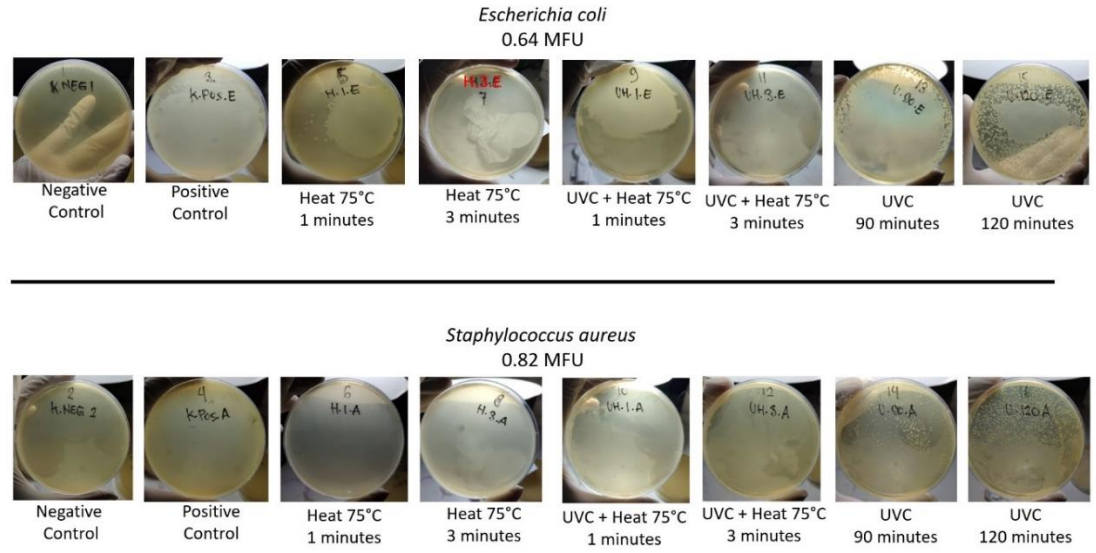

Figure 6. Mask culture results in shortened and extended sterilization durations

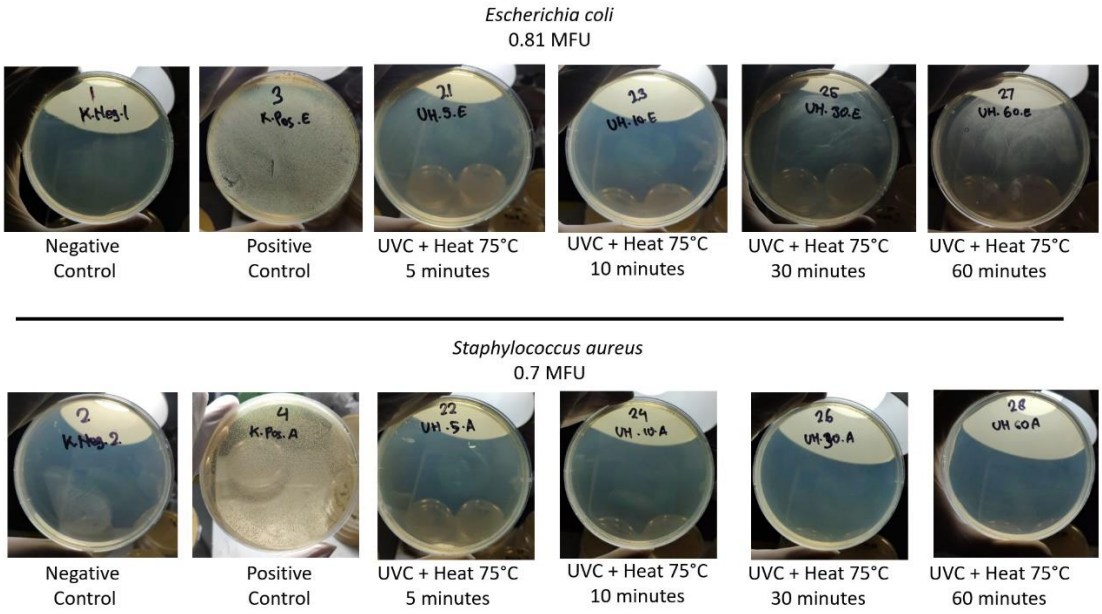

Figure 7. Mask Culture Results after Sterilization using both UVC and Heat

Aerosol Penetration and Air Resistance Test

The filtration ability of the N95 mask was maintained at >95% even though it had been through a sterilization process with 75°C heat, UVC, or a combination of both for up to 60 minutes Figure 8. In terms of air resistance, there was also no significant difference (ρ=0.07–0.50) between new masks and masks that had been sterilized using a portable sterilizer as shown in supplementary Figure 9.

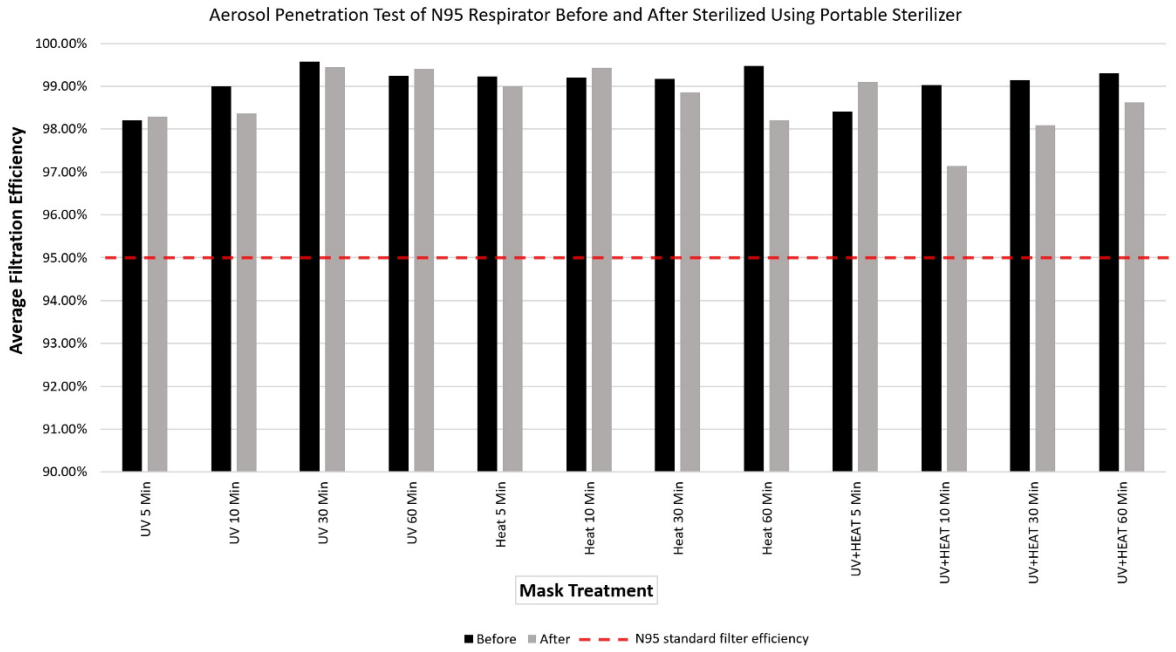

Figure 8. Comparison of N95 masks aerosol filtration efficiency before and after sterilization using the portable sterilizer

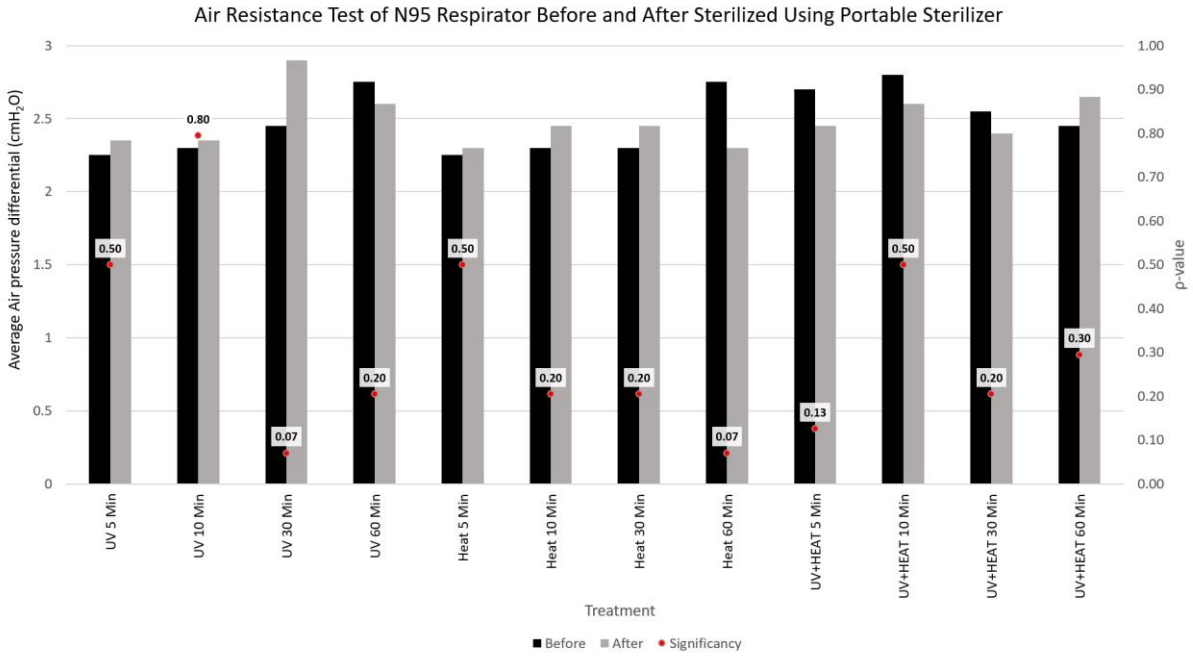

Figure 9. Comparison of Air Resistance of N95 Masks before and after Sterilization using the Portable Sterilizer

Based on the sterilization ability test results, using a portable sterilizer with the heat method was more effective in eradicating *S. aureus* and *E. coli*. This method killed the bacteria on the mask pieces starting by heating for 3 minutes, while UVC still gave positive culture results even though the mask pieces had been exposed to UV light for 2 hours. This result shows that the use of heat in the portable sterilizer is more effective when compared to the use of UVC rays. The combination of heat and UVC also gave a

negative result on the culture results. Thus there was no need to use both methods because it was just a waste of energy.

One of the factors that increase heat sterilization capability is humidity. The use of moist heat is more effective than the use of dry heat.[16] Humidity in tropical countries like Indonesia is relatively high. In this experiment, the humidity level in the room was around 55–60% RH. This high humidity environment increased the effectiveness of the heat sterilization capability of this portable sterilizer without the need for modification of the humidity level in the sterilization chamber. Other studies have also shown that heat is more effective than treatment using UVC for the sterilization of N95 masks.[17]

Bacteria's walls composed of protein have thermophobia characteristics. High temperatures will cause denaturation of these proteins and result in the death of these microorganisms.[18] *E. coli* at 60°C will die within 2.9 minutes.[19] Meanwhile, *S. aureus* will die at a temperature of 60°C for about 4.8–6.6 minutes.[20] For comparison, heating containing *SARS CoV-2* media at 65°C for 3 minutes is recommended to kill the COVID-19 virus.[21] However, *Mycobacterium tuberculosis* needs a higher temperature at 80°C to lose its viability.[22] But, it should also be considered that too high temperatures can damage the structure of N95. The polypropylene layer on the N95 mask has a melting point of 130–171°C.[23] If the temperature is too close, the structure will be damaged, impacting its filtration performance. Heating at a temperature of 125°C can reduce the filtration ability of N95 up to 90%.[7] We had tried to use an autoclave for the initial sterilization process to remove the environmental contamination of the mask sample before it was artificially contaminated. However, the mask sample showed a physical deformity like melting after being removed from the autoclave. Although it has excellent germicide capabilities, using an autoclave was not recommended in sterilizing N95 masks.[24]

The filtration ability of N95 is obtained by utilizing a combination of polypropylene microfibre and electrostatic charges. The name N95 was given because this mask could filter at least 95% of solid and aerosol particles in laboratory trials. The letter N indicates that this mask cannot filter oil-based vapor.[25] The N95 mask consists of several layers, one of which is a layer made of nonwoven polypropylene fiber with a diameter of 4.2 ± 3.9 µm, forming a layer with a thickness of 200-400 µm.[26,27] The sterilization process must maintain the electrostatic charge of this membrane so that the mask filtration performance does not decrease.

Golovkine et al. showed a decrease of 3 log concentrations of *SARS CoV-2* on the N95 surface after sterilization using UVC light at 1 mW/cm^2 for 10 minutes.[28] This result could be achieved because the UVC light source they used was very close and right on the mask's surface and back, leading to an adequate UVC exposure. In contrast to this portable sterilizer, the lack of effectiveness UVC is thought to be the result of the device configuration. The mask was placed in a hanging position on the side of the sterilization chamber, while the UV light source only came from above, resulting in uneven exposure to UVC rays and a blind spot in the sterilization process. In addition, this study did not measure the UVC radiation exposure dose, so that the UVC dose may be too low. However, exposure of the mask to UVC rays at an excessive dose can also reduce the filtering performance of the mask; thus, a precise dose is required.[29]

This sterilizer was designed because the heat source was located directly at the bottom of the sterilization chamber. If the mask was placed directly under the UVC source, in the bottom position, it was feared that the mask would melt because it was so close to the heating source. Thus, the mask must be hung on the side. However, the advantages of this configuration make this tool capable of loading a total of four masks in one

sterilization process, making it more efficient in operation.

In terms of an aerosol penetration test, using a portable sterilizer did not reduce the mask's filtration performance below 95%, whether using UVC, heat, or a combination of both, even after being exposed for 1 hour. This study also showed no significant change in the air resistance of the N95 mask in all sterilization methods for 1 hour, so the user was still comfortable breathing when using a sterilized mask. Another study also provided a similar result. Xiang Y *et al.* reported that exposure to dry heat to N95 at a temperature of 60°C and 70°C for 1 hour killed seven strains of bacteria and fungi, including *E. coli* and *S. aureus,* without reducing their filtration ability below 95%.[30] Even the use of heat up to 100°C for 5 minutes repeated 20 times did not affect the filtration ability of the N95 mask. [7]

CONCLUSIONS

It can be concluded that this portable sterilizer was able to kill *E. coli* and *S. aureu*s in the N95 mask using the 75 °C heat method for 3 minutes without negatively affecting the filtration performance and air resistance.

Although the effect was shown starting from 3 minutes, we recommend using this portable sterilizer with the heat method with a minimum duration of 5 minutes to compensate for the time that this tool takes to raise the temperature from room temperature (25 °C) to operational temperature (75°C). In addition, this mini sterilizer is only for emergencies, such as when there is a shortage of N95 masks. However, it is much safer to use a new mask than a mask that has undergone sterilization.

The advantage of this tool is that it is smaller, compact, portable, and easy to use compared to the tools used in previous studies, which mainly used tools that were generally used on a commercial scale. The design itself still needs much improvement. Form mask placement needs to redesign, so it is safe from heat sources. Besides, the operating system needs to be changed from analog to digital so that the timer set becomes easier to set, and the exterior design must be updated to make it look contemporary. In addition, many more tests are needed regarding the effect of using a mini sterilizer on N95 masks, such as the impact of repeated use on masks, its effect on microscopic N95 fibers, the elasticity of mask strap rubber and fitment test, calculation of UVC doses, and the effect on various airborne pathogens such as *M. tuberculosis* and *SARS CoV-2.*

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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