

Sterilizing personal protective equipment using UV-C radiation in idle biosafety cabinets: A potential method to ease the burden during times of exceptional stress on the healthcare system

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ABSTRACT

DISCLAIMER: This article does not represent the official recommendation of the Cleveland Clinic or Case Western Reserve University School of Medicine, nor has it yet been peer reviewed. We are releasing it early, pre-peer review, to allow for quick dissemination/vetting by the scientific/clinical community given the necessity for rapid conservation of personal protective equipment (PPE) during this dire global situation. We welcome feedback from the community.

Personal protective equipment (PPE), including surgical masks and N95 respirators, is crucially important to the safety of both patients and medical personnel, particularly in the event of infectious pandemics. As the incidence of Coronavirus Disease (COVID-19) is currently increasing exponentially in the United States and worldwide, healthcare provider demand for these devices is currently outpacing supply. As such, strategies to safely expand the lifespan of the current supply of medical equipment is of great importance. In this study, we outline a procedure by which N95 respirators may be sterilized in biosafety cabinets, a staple element of many academic, public health, and hospital laboratories using ultraviolet radiation. To ascertain heterogeneity in spatial dosing, we tested the UV-C radiation among a subset of the biosafety cabinets (BSCs) in our research institute and showed that the spatial variance in dose at the base of the BSCs varied by a factor of 1.53. Based on these values, we calculated that an N95 mask placed within a biosafety cabinet with a manufacturers reported fluence of $100 \mu W cm^{-2}$ could be effectively sanitized for reuse after approximately 15 minutes per side. Our results provide support to healthcare organizations looking for alternative methods in which to enhance the longevity of their supply of personal protective equipment. It is our hope that with an easy to implement strategy, as we have presented here, unused BSCs can be utilized to alleviate the supply shortage of PPEs by providing a safer way to reuse masks compared to continued daily use of masks.

Introduction

Personal protective equipment (PPE) is essential for protecting medical personnel and patients during outbreaks of airborne infectious diseases. In particular, the use of surgical masks and N95 respirators are recommended for infections that may be transmitted by respiratory droplets and airborne particles, respectively. Due to the rapidly emergent nature of the novel Coronavirus Disease (COVID-19) and stringent requirements of proper PPE protocol,

many hospitals are running dangerously low on these respiratory protection devices. As a result, both patients and their healthcare providers are at increased risk of contracting and spreading COVID-19.

One consequence of the rapid spread of COVID-19 is that the manufacturing of respiratory protective equipment is not currently meeting the exponentially growing demand, creating a supply shortage. As many scientists in the community have previously suggested, one method of preserving our current supply of N95 filtering face-piece respirators (FFRs) is through cycles of decontamination and reuse with ultraviolet germicidal irradiation.¹⁻⁵ Ultraviolet (UV) light is a form of electromagnetic radiation with more energy than visible light, but less energy than x-rays. It can be categorized into UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm) light. Ultraviolet germicidal irradiation (UVGI) uses UV-C at 254 nm. The higher energy UV-C rays have the ability to damage DNA and RNA via cross-linking of thymidine and uracil nucleotides, respectively, thus preventing the replication of microbes such as bacteria and viruses.⁶ At these wavelengths, the amount of microbial inactivation is directly proportional to the dose of UV radiation, with dosage being defined as the product of intensity (W/m^2) and exposure duration (s). Therefore, UVGI is a relatively simple method of sanitation with minimal damage to the respirator and avoids use of irritating chemicals.

Though there is no current consensus on the amount of UV radiation required to inactivate SARS-CoV-2, the UV dose required to inactivate 90% of single-stranded RNA viruses has been estimated to be $1.32 - 3.20 \text{ mJ/cm}^{-2}$,¹ and similar methods using 254 nm UVC light have been investigated with SARS-CoV-1.⁷ A group at the University of Nebraska Medical Center recently developed a protocol to sterilize N95 respirators using UVGI.⁸ Specifically, they subjected used N95s to 60 mJ/cm^{-2} of UVC radiation (254 nm)—which exceeds the estimated sterilization dose by several-fold—by stringing them across a room that contained two UVGI towers on either side. They then remotely monitored the effective UVGI dose on the respirators using a UV meter to ensure proper sterilization.⁸

Unfortunately, not all hospitals are equipped to set up dedicated rooms for decontamination or possess the specialized UVGI towers that are outlined in the Nebraska protocol. On the other hand, many university-affiliated hospitals and BSL2 or higher academic laboratories have access to biosafety cabinets that are regularly used in research to sterilize microbes via UV-C light. Due to current social distancing measures, the majority of these biosafety cabinets are not currently in use and therefore can be repurposed to irradiate N95 respirators. A previous study found that UVGI treatment of FFRs in biosafety cabinets had no effect on the filter aerosol penetration, filter airflow resistance, or physical appearance of the masks.⁴ Additionally, the efficacy of biosafety cabinet UVGI for achieving complete decontamination of FFRs has previously been validated for influenza virus.⁹

Given the current pandemic situation of COVID-19, we sought to understand the efficacy of UVGI as a method

of sanitation to preserve the dwindling supply of FFRs. First, we measured the minimum light intensity output by a standard biosafety cabinet, as well as the variability of light intensity between several biosafety cabinets. From these measurements, we calculated a recommended time to irradiate FFRs in a biosafety cabinet to inactivate potential SARS-CoV-2 virus contaminants. Finally, we estimated the potential number of FFRs per day that can be sanitized in the United States using this protocol.

Methods

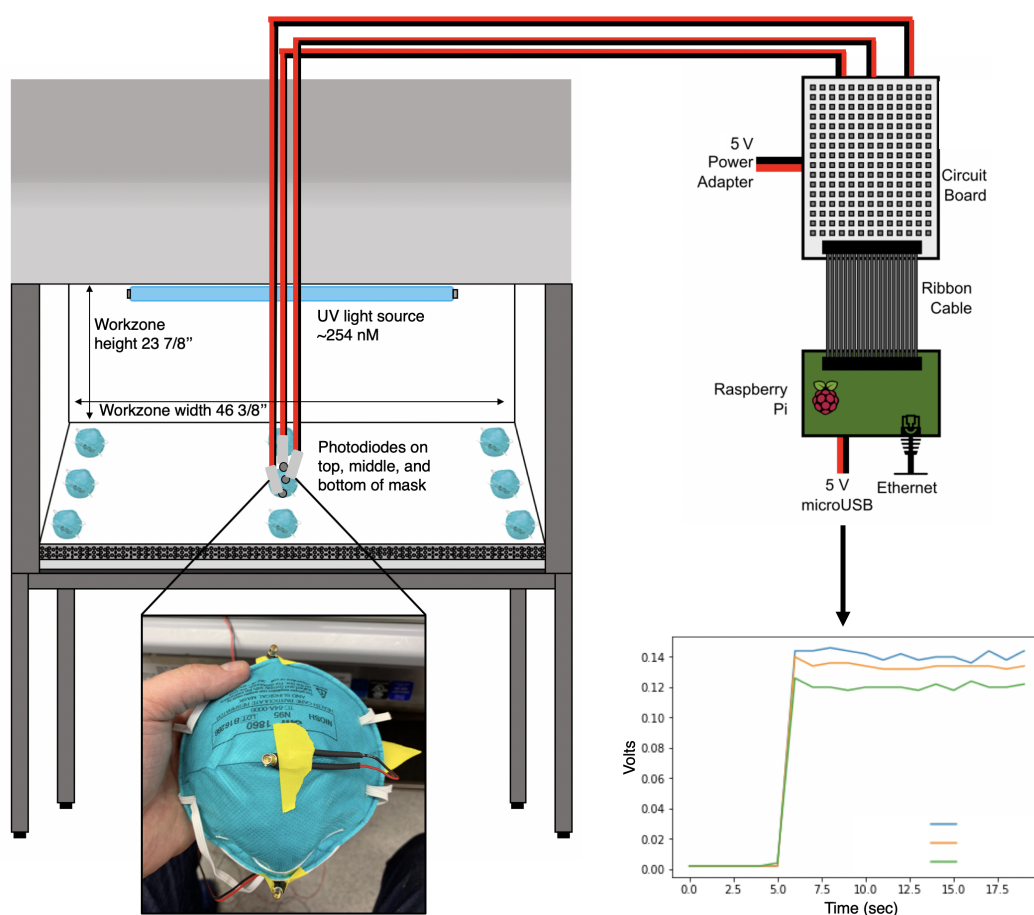


Figure 1. Schematic of our process for measuring light intensity across a biosafety cabinet. A photodiode was attached to the top, middle, and bottom of an N95 mask, and the voltage of light that reached diodes was measured both with the UV light turned off and then on. This measurement was performed within each sector of a 3x3 grid at the base of the biosafety cabinet workzone as illustrated in schematic.

The biosafety cabinets used in this experiment were LabGard ES NU-425 Class II, Type A2 models (NuAire, Plymouth, MN), which are reported to use 253.7 nm UV-C radiation and provide an average intensity of $100 \mu\text{Wcm}^{-2}$. We affixed three photodiodes (MTPD4400D-1.4) to a standard N95 respirator (3M) and measured UV fluence from nine positions (in a 3x3 grid) equally spaced on the counter of each biosafety cabinet (Fig 1).

Measurements of light intensity from the photodiodes were continuously recorded by a Raspberry Pi at a resolution of 40ms for 4 seconds. A circuit board with an LM324N operational amplifier (for signal amplification) and an ADS1015 analog to digital converter were used to interface the photodiodes and the Pi. The lowest measured intensity was recorded for each cabinet, and the variance in minimum intensities between cabinets was determined.

Resulting data was used to generate heatmaps of the mean values from all three photodiodes at each position of the 3x3 grid at the base of the biosafety cabinets. Analysis was performed in the R programming language. Then, the time required to deliver a dose of 60mJ/cm² was calculated by dividing the desired UV dose by the minimum intensity.

Results

UV Intensity among existing biosafety cabinets

The technical specifications of the BSC suggest that approximately 100 μWcm^{-2} of 254nm UV-C radiation are received along the floor of the cabinet. However, given the geometry, we were concerned about spatial heterogeneities in this delivery and realized that masks in different positions could receive different doses. To understand the magnitude of this heterogeneity we designed an experiment to explore the spacial heterogeneity across BSC workzone base and over different parts of the mask. Using an array of photodiodes attached to a standard N95 mask (see methods), we assayed the heterogeneity along the bottom of the cabinet. From these measurements, we derived an upper and lower bound estimate for each 3x3 grid at the bottom of the BSCs (Fig 2). To do this we took the average of the three photodiodes per array – we used the mean as we consider the photodiodes to be technical replicates with positional variation. Using the upper bound (the highest measured UV fluence in the 9 sections), we assumed that this value corresponded to the manufacturers reported dose. Then, to be conservative, we made our estimate of time needed for sterilization based on the lowest measured dose in the 9 sectors.

Relative UV intensity as a function of spatial position at the base of two BSCs. Each of the nine sections denoted in each hood contains the data from three photodiodes attaches to the surface of an N95 mask. The minimum intensity measured among the two biosafety cabinets measured was a factor of 1.54 below the maximum. The mean intensity across all measurements with the UV lights on was 0.11, the range was 0.08 - 0.16, and the standard deviation was 0.02. Given the manufacturers published fluence of 100 μWcm^{-2} , which we conservatively presume to correspond to our highest measurement, and the Nebraska estimate of 5 min for sterilization at 200 μWcm^{-2} , we estimate that 15 minutes (per side) are required for the level of sterilization published by the Nebraska group in the cabinets we tested.

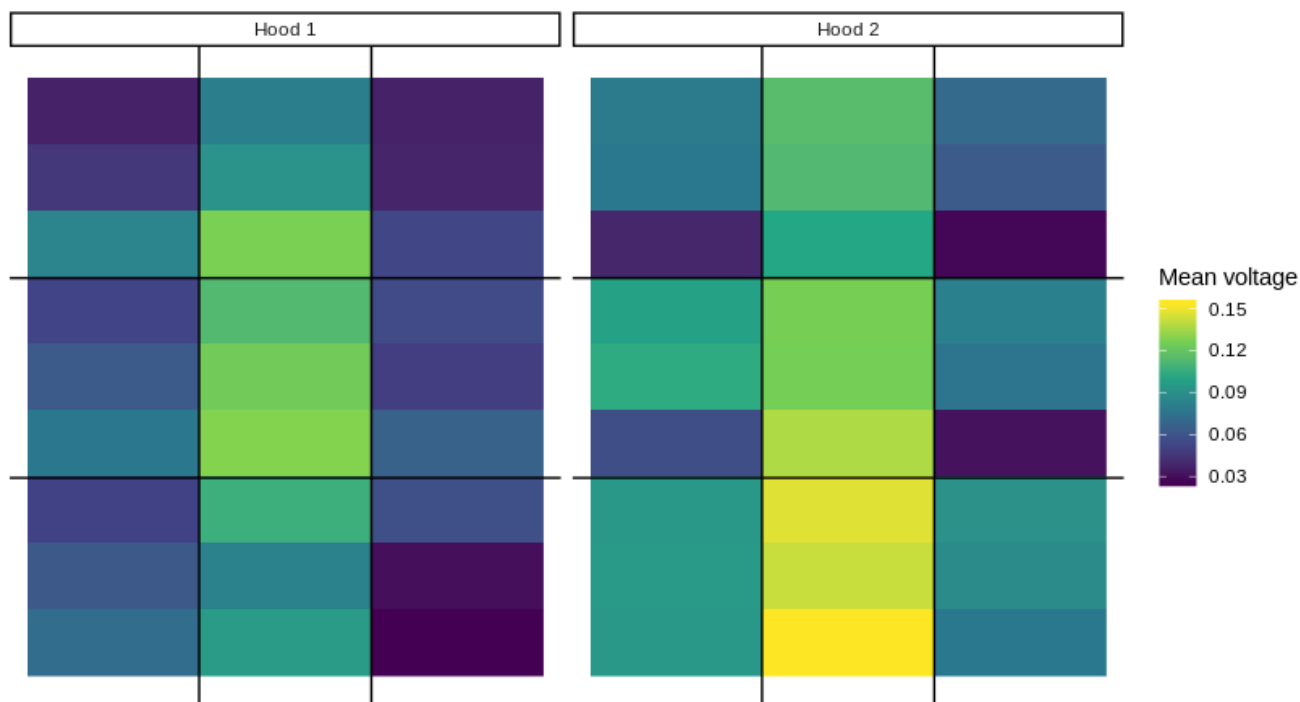


Figure 2. Relative UV intensity as a function of 2D position at the base of two BSCs. Each of the nine sections per hood contains the data from three photodiodes attached to the surface of an N95 mask. The three photodiodes were attached to different positions on the mask (Fig 1) to demonstrate UV differences across mask surface. The mean of each grid sector was measured and used in calculations to determine minimum time of mask exposure for sterilization.

For other cabinets, a similar scaling law could be derived based on our measured variance in fluence across the cabinet floor. We would recommend a similar method, scaling time by the manufacturers reported fluence, the estimates published by the Nebraska group, and the heterogeneous spatial distribution of dose. Therefore, conservatively, we recommend 1.5x the time per side in a BSC, multiplied by the ratio of the manufacturers published fluence to the published Nebraska value of $200 \mu W cm^{-2}$.

Estimating the impact of using UVGI to sanitize FFRs in the United States

Due to the lack of concrete data on the number of currently available biosafety cabinets in the United States, we will use the tried and true “Fermi method” to estimate the number of FFRs that can be re-sanitized using the method outlined above. The Fermi method, named after the renowned physicist Enrico Fermi, uses a sequence of “best guess” estimates that are ultimately multiplied together to reach an overall approximation. The error associated with a Fermi estimate is roughly $10^{\sqrt{n}}$, where n is the number of “best guess” estimates made.^{11,12} Given these considerations, our estimates are outlined below.

At the present time there are roughly 83,000 biological scientists in the United States (<https://datausa.>

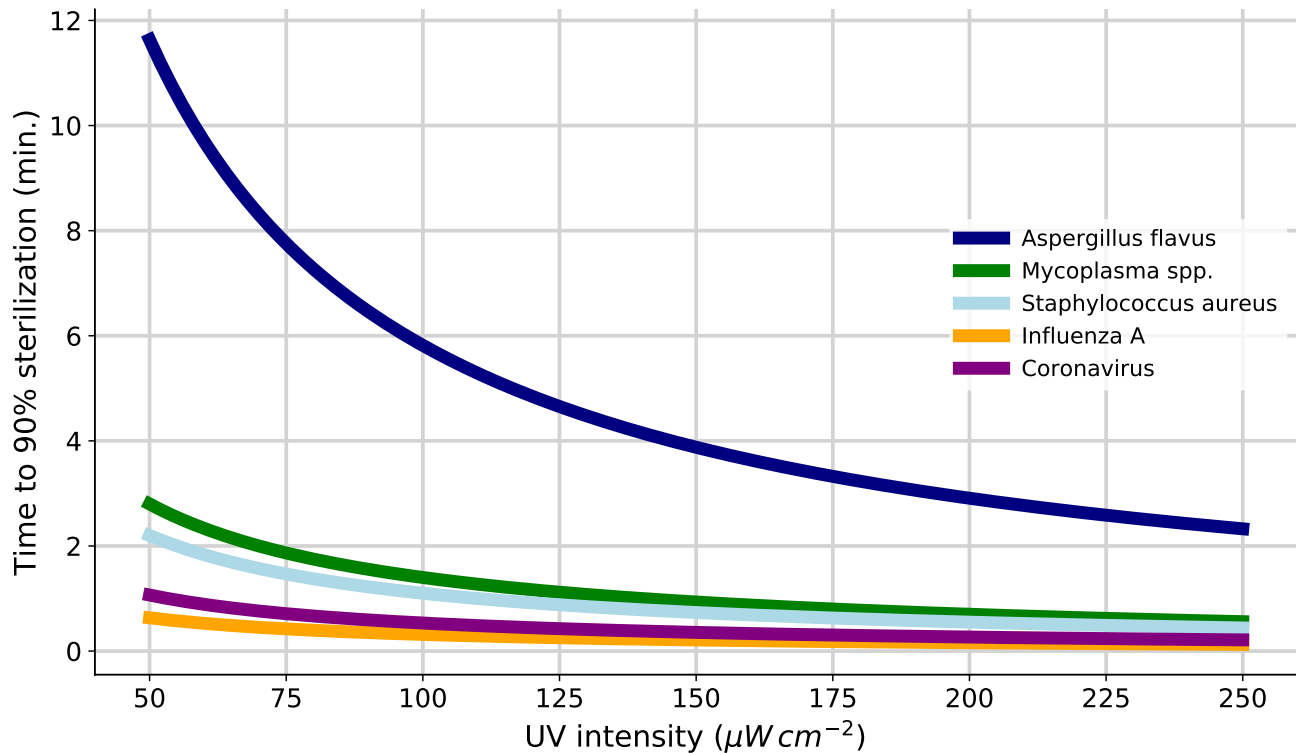


Figure 3. Time in minutes to reach 90% sterilization for a given UV intensity, shown for various representative pathogens.^{8,10}

[io/profile/soc/biological-scientists](#)). If roughly half of these scientists have access to biosafety cabinets, and the ratio of cabinets to scientists is estimated at 1 : 10, then this suggests that roughly 4,000 biosafety cabinets are available in the United States. Most of these biosafety cabinets are idle at this time, given the restrictions placed to limit the spread of COVID-19.

It has been shown that three sanitization procedures of $18 kJ m^{-2}$ applied over 15 minutes does not drastically impact the function of FFR.⁹ Accordingly, we assume that each FFR may be sanitized and reused three separate times. Given the importance of FFR in combating COVID-19, we assume that sanitization procedures will be implemented effectively nonstop. If each sanitization procedure takes 30 minutes, and 28 FFRs can be processed in each procedure, then the number of FFRs that can be recycled in one day by a single institution is:

$$\frac{24 \text{ hours}}{\text{day}} \times \frac{2 \text{ procedures}}{\text{hour}} \times \frac{28 \text{ masks}}{\text{procedure}} \times \frac{M \text{ BSC}}{\text{institution}} = \frac{1344 \text{ masks}}{\text{day}} \times M$$

where each of the M BSCs available per institution is running a simultaneous sanitization procedure – more than

1,000 N95 masks per day for every BSC if run at capacity.

The WHO estimated that roughly 89 million FFRs are needed in the response to COVID-19 per month¹³. In order to meet these demands, the worldwide production would need to increase by 40%. However, we estimate that nearly 162 million masks per month ($1344 \times 4000 \times 30$) can be sanitized for re-use under ideal conditions. Finally, because FFR masks can be UVGI sanitized at least three times without loss of function⁹, our calculations suggest we can lower FFR requirements by 75%.

Discussion

Ideally, a new mask or respirator would be used for each individual to minimize the transmission of infectious diseases that are airborne or transmitted via respiratory droplets. However, crises such as the current COVID-19 pandemic can create shortages that necessitate measures to conserve PPE. Various groups have therefore begun sterilizing respiratory protective equipment themselves using UVGI and “homebrew” setups. For example, enterprising clinicians at the University of Nebraska Medical Center are stringing N95 respirators between two towers of UVGI bulbs placed on either side of a room in order to inactivate potential SARS-CoV-2 viral contaminants on the masks.⁸

From our measurements normalized to the technical specifications of the manufacturer using a typical biosafety cabinet, we estimate the time to sterilize N95 respirators using similar models is 15 min per side. This conservative recommendation represents the time required to deliver a UV dose that is ≈ 30 times higher than the previously reported dose to inactivate 90% of single-stranded RNA viruses.¹

In the future, it may be possible to design a technique that avoids the need to flip masks over and irradiate each side separately. By elevating masks off the floor of the biosafety cabinet and, if necessary, placing reflective material underneath them it should be possible to ensure that UV radiation reaches the entire mask surface simultaneously and would reduce the hands on aspect and time required for this protocol.

Drawing influences from the protocol developed by the University of Nebraska Medical Center,⁸ we propose a workflow to optimize the utilization of institutional resources. Prior to use, respirators should be labeled to identify the original owner by both name and department. After use, place in sealed packaging and distribute to biosafety cabinet locations. Using sterile technique, remove masks from packaging and place on working surface of cabinet. It is critical to ensure that there is no overlap of adjacent masks as any unexposed areas will not be sterilized. After transfer, adequately sterilize any external surface that came in contact with the used masks or packaging and destroy the packaging via biological waste. Close the hood and power on the UV light for 15 minutes. After this duration, power off the UV light, open the cabinet, and carefully flip the masks to expose the opposite side. Again, ensure no overlap of adjacent masks. Close the hood and power on the UV light for 15 minutes. Again, adequately sterilize or

dispose of any external surface that comes in contact with the masks. Once the full duration has completed, power off the UV light and open the hood. While maintaining sterility of the cabinet, add a tally to each mask indicating the number of UVGI cycles and individually place in sterile, sealed packaging. Remove packages from cabinet and redistribute to original owner.

Limitations

Despite the measures taken here to err on the side of overestimating the recommended time for irradiation of N95 respirators, following this protocol by no means guarantees complete decontamination. For example, FFRs contain multiple layers of filtration, and respiratory droplets may penetrate into the inner layers. Though UV-C light has been shown to transmit into and through FFR materials, the transmittance of light ranges from 23-50% through the outer layer depending on the model of FFR.⁵ Therefore, the ability for UVGI to thoroughly sanitize FFRs may vary based on the ability for UV-C light to penetrate through to the internal filtering medium, which contributes the most filtration ability.

Another concern of reusing N95 respirators is the UV-mediated degradation of polymers within the respirator. The maximal number of decontamination cycles has been suggested to be determined by the physical degradation of the respirator material, rather than the loss of filtration capacity.³ As such, we recommend that hospitals employing this approach take additional precautions such as: 1) labeling N95 respirators so that they can be reused by the same individual, and 2) marking the number of times the same mask has undergone decontamination, as was recommended by our colleagues in Nebraska.⁸ In contrast to the eventual degradation of the respirator material, Ref.³ found that the straps retained their structural integrity even at high UV doses. This would be crucial to maintaining the tight fit of the mask through repeated decontamination cycles. Though the situation that necessitates it is far from ideal, using presently-unused biosafety cabinets to sanitize N95 respirators has the potential to reduce the current PPE burden by 75%.

1 Code and Data Availability

All data used in this paper and code written to analyze it are open source and publicly available.¹⁴

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Author contributions statement

This was a massive team effort with everyone contributing their specific expertise. All listed members of the team contributed to the writing of the manuscript.

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