UV Sterilization of Personal Protective Equipment with Idle Laboratory Biosafety Cabinets During the COVID-19 Pandemic.

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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 increasing exponentially in the United States and worldwide, healthcare provider demand for these necessities is currently outpacing supply. As such, strategies to safely expand the lifespan of the supply of medical equipment are critically important. In the recent days, weeks, and months, in the midst of the current pandemic, there has been a concerted effort to identify viable ways to conserve Personal Protective Equipment, including sterilization after use. Some hospitals have already begun using UV-C light to sterilize N95 respirators, but many lack the space or equipment to implement existing protocols. In this study, we outline a procedure by which N95 respirators may be sterilized using ultraviolet (UV) radiation in biosafety cabinets (BSCs), a common element of many academic, public health, and hospital laboratories. The primary obstacle to this approach is the possibility the UV radiation levels vary within BSCs. To account for this potential variation in dosing across the base of the BSC, we tested the UV-C radiation in two randomly chosen idle BSCs in our research institute and observed a maximum ratio between the minimum and maximum recorded intensities within a given BSC to be 1.98. Based on these values, we calculated that an N95 mask placed within a BSC with a manufacturer's reported fluence of 100 $\mu W \mathrm{cm}^{-2}$ should be effectively sanitized for reuse after approximately 15-20 minutes per side. Our results provide support to healthcare organizations looking for alternative methods to extend their reserves of PPE. It is our hope that with an easily implemented strategy, as we have presented here, idle BSCs can be utilized to alleviate the PPE shortage by providing a way to sterilize PPE to allow safe daily re-use. This should be tested on a larger scale, and confirmed in a virology laboratory before adoption, though we contend that in extremis, this method would be preferred compared to re-use without sterilization.

Introduction

Personal protective equipment (PPE) is essential for protecting medical personnel and patients during outbreaks of airborne or droplet borne infectious diseases. In particular, the use of surgical masks and N95 respirators is

recommended for infections that may be transmitted by respiratory droplets and airborne particles, respectively.[1] 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 protective devices. As a result, both patients and their healthcare providers are at increased risk of contracting and spreading COVID-19.

As 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 (UVGI).[2–6] 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). UVGI uses UV-C within 200-300 *nm*.[7] The higher-energy UV-C rays can damage DNA and RNA via cross-linking of thymidine and uracil nucleotides, respectively, thus preventing the replication of microbes such as bacteria and viruses.[8] At these wavelengths, the amount of pathogen 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).[9,10] Therefore, UVGI is a relatively simple method of sanitation that causes minimal damage to the respirator and avoids the use of irritating chemicals.

Although there is no current consensus on the amount of UV radiation required to inactivate SARS-CoV-2, the single-stranded RNA (ssRNA) virus that causes COVID-19, the UV dose required to inactivate 90% of ssRNA viruses is an estimated $1.32 - 3.20$ mJ cm^{-2}.[2] Similar methods using 254 nm UV-C light have been investigated with SARS-CoV-1.[11] A group at the University of Nebraska Medical Center recently developed a protocol to sterilize N95 respirators using UVGI.[12] Specifically, they subjected used N95s to 60 mJ cm^{-2} of UV-C radiation (254 nm)—which exceeded the estimated sterilization dose of 2-5 mJ cm^{-2} for single-stranded RNA by several-fold—by stringing them across a room containing two UVGI towers on either side. The UVGI doses were remotely monitored using a UV meter to ensure proper sterilization.[12]

Unfortunately, not all hospitals are equipped to set up dedicated rooms for decontamination or possess the specialized UVGI towers that are outlined by Lowe et al.[12] On the other hand, many university-affiliated hospitals and higher academic laboratories have access to biosafety cabinets (BSCs) that are regularly used in research to sterilize laboratory equipment via UV-C light. Due to current social distancing and quarantine measures, there likely exist a substantial number of BSCs that are not currently in use and therefore may be available to be temporarily repurposed for N95 respirator, or other PPE decontamination. A previous study found that UVGI treatment of FFRs in BSCs had no effect on the filter aerosol penetration, filter airflow resistance, or physical appearance of the masks.[5] Additionally, the efficacy of BSC UVGI for achieving complete decontamination of FFRs has previously been validated for influenza virus.[13]

Given the urgency of the ongoing COVID-19 pandemic, we sought to determine if BSCs could be temporarily repurposed for UVGI disinfection to preserve a dwindling supply of FFRs. To do this, we measured the minimum light intensity output by a standard BSC, as well as the variability of light intensity between several BSCs. From these measurements, we calculated a recommended time of 15-20 minutes per side to irradiate FFRs in a BSC to inactivate potential SARS-CoV-2 virus contaminants. Due to the large number of idle BSCs nationwide, we estimate FFR requirements can be reduced by up to 75%, significantly easing hospital burden due to a significant lack of PPE.

Methods

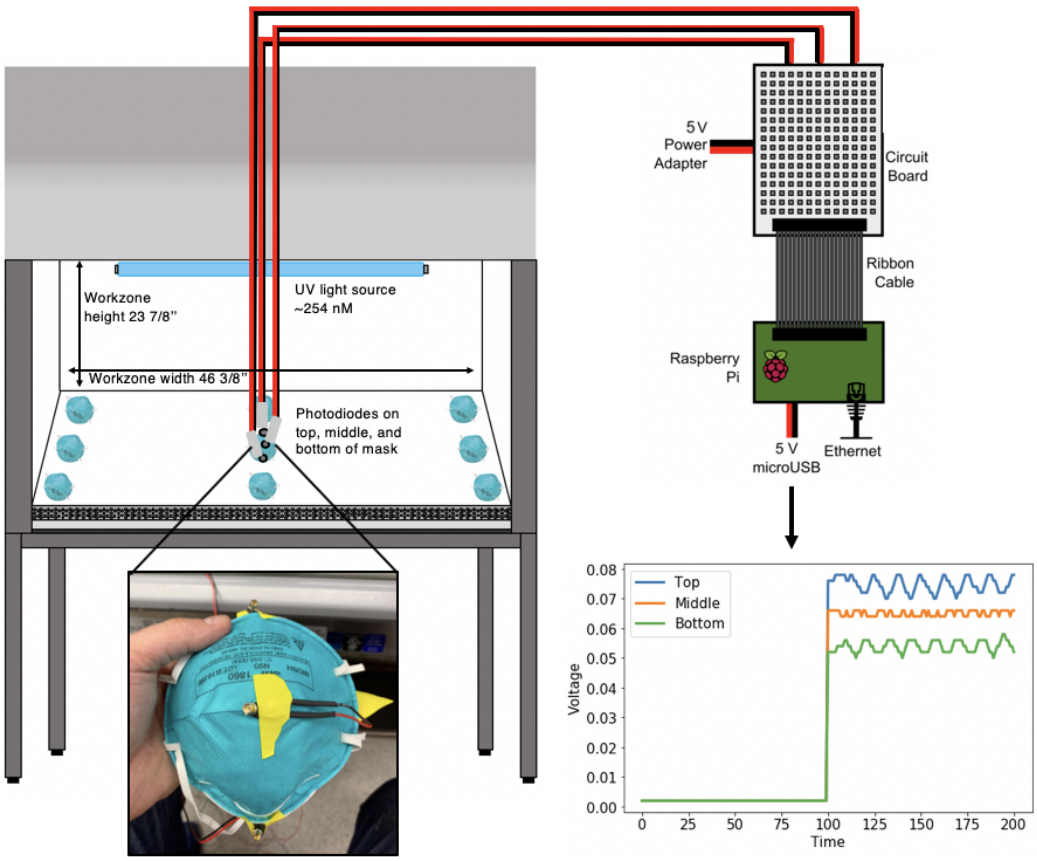

Figure 1. Schematic of our process for measuring light intensity across the base of a BSC. A photodiode was attached to the top (north), middle, and bottom (south) of an N95 mask, and the voltage of light that reached diodes was measured both with the UV light turned off and then on. This measurement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

The BSCs used in this experiment were LabGard ES NU-540-400 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 $\mu W \text{cm}^{-2}$ to the cabinet floor. We affixed three photodiodes (MTPD4400D-1.4) to a standard N95 respirator (3M) and measured UV

fluence from nine positions (across a 3x3 grid) equally spaced on the counter of each BSC (Fig 1). Measurements of light intensity from the photodiodes were recorded by a Raspberry Pi at 40ms intervals for a total period of 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.

Resulting data were used to generate heatmaps of the values from all three photodiodes at each position of the 3x3 grid at the base of the BSCs. Analysis was performed in the R programming language[14] using the ggplot2[15] and dplyr[16] packages (all code and data may be viewed at https://github.com/TheoryDivision/covid19_biosafety_cabinet[17]). Then, the time required to deliver the dose of 60mJ/cm^2 was calculated by starting with the 5 minutes determined by Lowe et al, adjusting for the difference in UV light fluence (per manufacturer's records), and applying a scaling factor based on the difference between the highest and lowest recorded intensity within the grid.[12]

Results

UV intensity among existing BSCs

The technical specifications of the BSC suggest that approximately 100 $\mu W \text{cm}^{-2}$ of 254nm UV-C radiation is received along the floor of the cabinet. From the reported UV intensity of a BSC (or any UV-C source) and measured lethal doses for ssRNA viruses and other microbial pathogens in the literature, one can estimate the time to sterilization, assuming insignificant decay in intensity due to equipment wear over time, homogeneous UV irradiance across the source, homogeneous radiant flux over the area of the mask, and comparable pathogen survival between media tested in literature and colonized N95 mask (Fig 3).

However, given the geometry of the mask, we were concerned about spatial heterogeneities in this delivery and hypothesized that masks in different positions could receive different doses. To understand the magnitude of this heterogeneity, we designed an experiment to explore the spatial heterogeneity across a BSC workzone base and over different parts of the mask. Using an array of photodiodes attached to a standard N95 mask (see Fig 1), 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 median of the three photodiodes per array (we used the median because 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 manufacturer's 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.

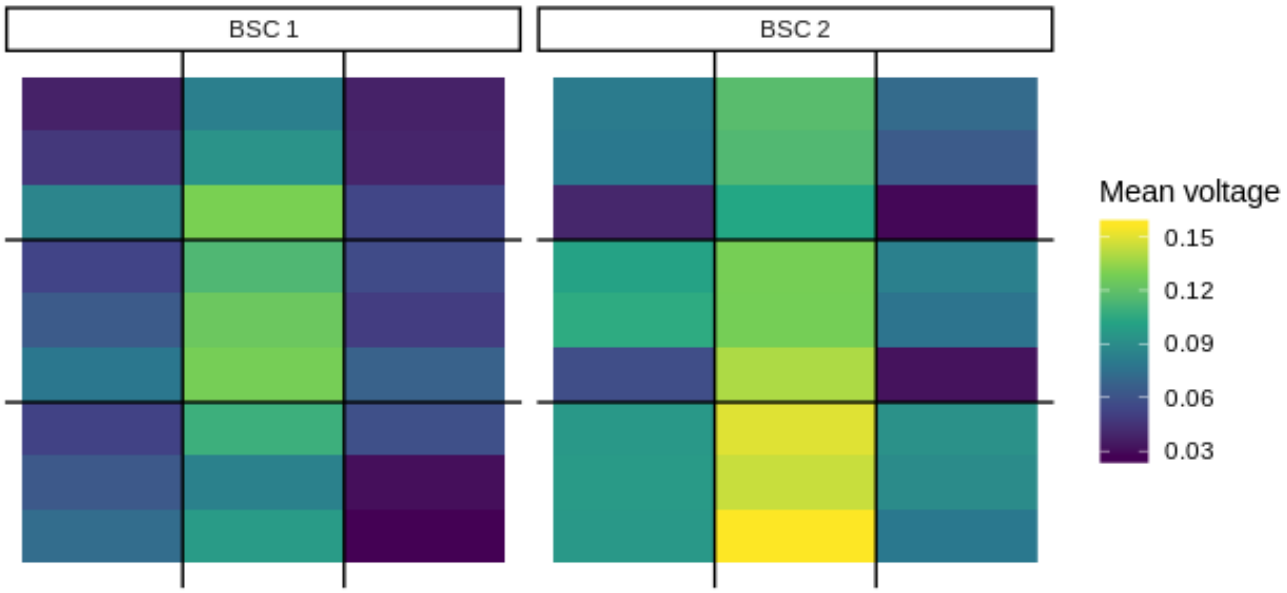

Figure 2. Relative UV intensity as a function of position across the base of two BSCs. Each of the nine sections per BSC shows the voltage from three photodiodes attached to the surface of an N95 mask (top, middle, and bottom). The three photodiodes were attached to different positions on the mask (Fig 1) to demonstrate UV differences across mask surface. To account for ambient light, voltages recorded with the UV lights off were subtracted from the voltages recorded with the UV lights on. The median of each grid sector was calculated and used in calculations to determine minimum time of mask exposure for sterilization.

Calculating a time to sterilization scaling factor using relative UV intensity as a function of spatial position at the base of two BSCs

Each of the nine BSC workzone positions contain data from three photodiodes attached to the surface of an N95 mask. The mean intensity across all measurements with the UV lights on was 0.11, with range 0.08 - 0.16, and standard deviation 0.02. To be conservative, we assume the largest measured intensity matches the manufacturer's reported value. Using this value, we then calculate UV received by the mask in the position of least intensity. The minimum measured intensities were a factor of 1.71 and 1.98 lower than the BSC 1 and BSC 2 maximums, respectively (see Table 1). To provide less conservative scaling factor estimates, we also calculate the median voltage for each section of the BSC 3x3 grid. The ratio between the maximum median intensity and minimum median intensity for each BSC are reported in Table 1.

Estimating time to sterilize FFR in a BSC

In addition to calculating a scaling factor to account for spatial heterogeneity across the BSC workzone, we must scale the estimated time to sterilization by a factor of 2 to incorporate the difference in fluence in Lowe et al.'s protocol (200 $\mu W \text{cm}^{-2}$) and the BSC used in our methods (100 $\mu W \text{cm}^{-2}$). Finally, we estimate the range of time

Table 1. Range of scaling factors calculated to account for spatial intensity heterogeneity across individual masks and throughout BSC workzone. The scaling factor required to estimate the minimum sterilization time using the BSC while accounting for heterogeneity of UV intensity each mask may receive. The greatest factor difference between the highest and lowest measured UV intensity was 1.98.

Method	BSC 1	BSC 2
Median max:min intensity	1.54	1.83
Absolute max:min intensity	1.71	1.98

estimates to sterilize FFRs in a BSC as 15.4-19.8 minutes per side. These values were calculated using the lowest and highest scaling factors from Table 1, respectively, using Equations 1 and 2. While we performed this correction with measurements from an uncalibrated photodiode sensor circuit, this would ideally be measured more precisely with a calibrated UV detector to obtain the exact scaling necessary to ensure the UV intensity can provide a sterilizing dose. These calculations are shown here.

$$5 \text{ minutes} \times 1.54 \times \frac{200\mu W\text{cm}^{-2}}{100\mu\text{Wcm}^{-2}} = 15.4\text{minutes} \tag{1}$$

$$5 \text{ minutes} \times 1.98 \times \frac{200\mu W\text{cm}^{-2}}{100\mu\text{Wcm}^{-2}} = 19.8\text{minutes} \tag{2}$$

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 BSCs in the United States, we implemented the "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 combined together to reach an overall approximation. 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.io/profile/soc/biological-scientists). If roughly half of these scientists have access to BSCs, and the ratio of cabinets to scientists is estimated at 1 : 10, then this suggests that roughly 4,000 BSCs are available in the United States. Most of these BSCs are idle at this time, given the restrictions on laboratory work in place to limit the spread of COVID-19.

It has been shown that three repeated sanitization procedures of 18kJm^{-2} applied over 15 minutes does not

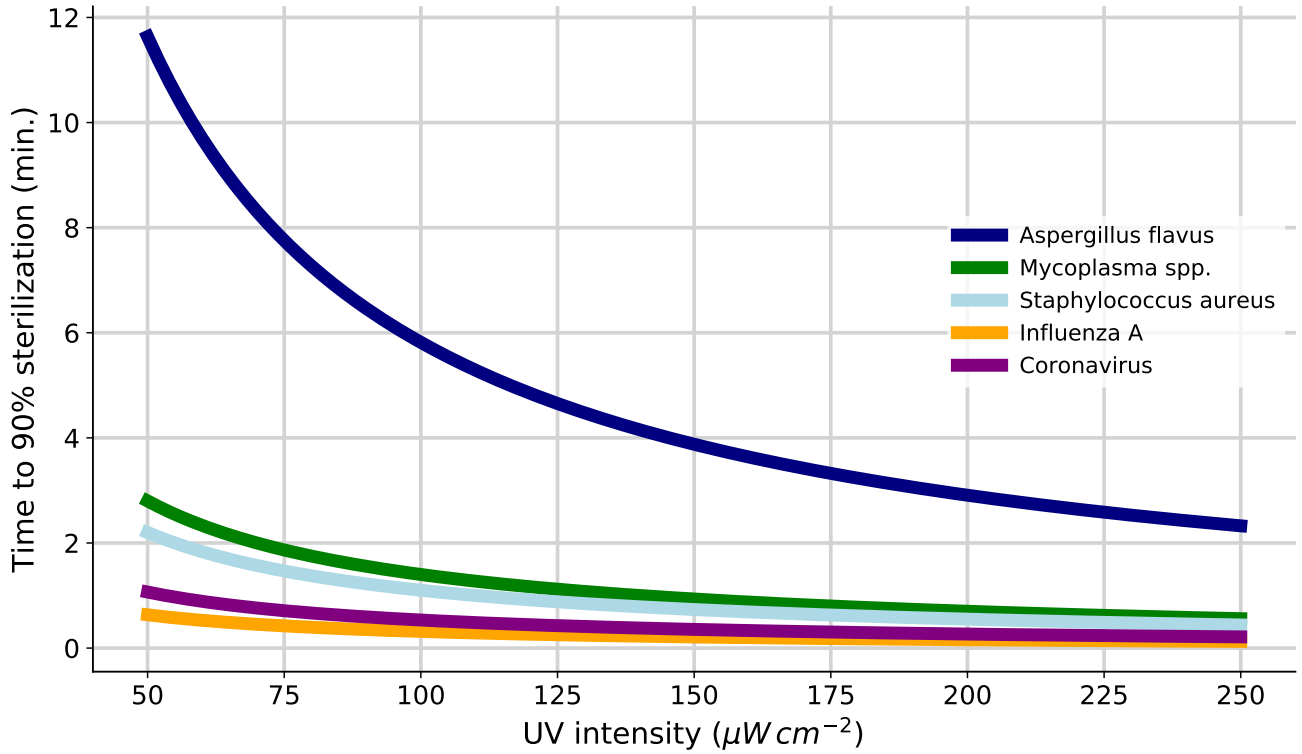

Figure 3. Time in minutes to reach 90% sterilization for a given UV intensity, shown for various representative pathogens. Using previously reported doses for 90% sterilization for key pathogens potentially, transmitted via respiratory droplets, time in minutes for 90% sterilization is shown for a range of UV intensities. [2,18]

drastically impact the function of FFRs.[13] 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 40 minutes (our most conservative time estimate), 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 calculated by equation 3:

$$\frac{24\text{ hours}}{\text{day}} \times \frac{1.5\text{ procedures}}{\text{hour}} \times \frac{28\text{ masks}}{\text{procedure}} \times \frac{M\text{ BSC}}{\text{institution}} = \frac{1008\text{ masks}}{\text{day}} \times M, \tag{3}$$

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 month.[19] In order to meet these demands, the worldwide production would need to increase by 40%. However, we estimate that nearly 121 million masks per month ($1008 \times 4000 \times 30$) can be sanitized for re-use under ideal conditions. Finally, because FFR masks can be UVGI sanitized at least three times without loss of function,[13] our calculations suggest

we can lower FFR requirements by 75%, from roughly 89 million FFRs per month to about 22 million FFRs per month.

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. Among potential methods for decontamination, previous work has suggested UVGI results in less physical deformation then bleach, microwave irradiation, and vaporized hydrogen peroxide.[5] Additionally, this and other investigation of UVGI for the purpose of N95 respirator decontamination was motivated by the ubiquity of UV lamp equipped biosafety cabinets, especially at large biomedical research institutions. 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.[12]

From our measurements, normalized to the technical specifications of the manufacturer using a typical BSC, we conservatively estimate the time to sterilize N95 respirators using similar models is 20 min per side. This 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.[2] To scale this protocol to any BSC, we propose that a conservative scaling factor of 1.98 for spatial heterogeneity is likely conserved across BSCs, but the amount of power delivered to the BSC surface likely varies significantly between manufacturers. Note that given the variance between the two BSCs we measured, the true worst-case scaling factor is likely slightly worse than what we measured, as there almost certainly exist BSCs with greater variation in UV radiation intensity. We invite other scientists to add measurements from their own BSCs to our github repository to allow continued updating of this recommendation.[17] Therefore, to calculate a time for an arbitrary BSC model, we recommend using Equation 4:

$$5 \text{ minutes} \times 1.98 \times \frac{200 \mu W \text{cm}^{-2}}{\text{unit} - \text{specific intensity}} = \text{recommended time.} \tag{4}$$

Note that this equation assumes that the UV radiation output of the BSC matches the manufacturer's specification. Ideally, BSCs should be calibrated before being used for the purposes of sterilizing PPE.

In the future, it may be possible to design a technique that avoids the need to flip masks over and irradiate

Value	Description
5 minutes	Time to reach germicidal exposure (60 mJ cm^{-2}) at power of 12 mJ/min determined by Lowe et al[12]
1.98	Most conservative spacial intensity scaling factor, per Table 1
200 $\mu W cm^{-2}$	Intensity of 254 nm UV light used by Lowe et al[12]
unit-specific intensity	Manufacturer-specified or measured UV-C intensity at the surface of the BSC[20]
recommended time	Estimated time to decontaminate one side of an FFR

each side separately. By elevating masks off the surface of the BSC 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 manual labor and time required for this protocol.

Inspired by the protocol developed by Lowe et al, we propose a workflow to optimize the utilization of institutional resources:[12]

1. Prior to use, respirators should be directly labeled to identify the original owner by both name and department.
2. After use, place in sealed packaging and distribute to BSC locations.
3. Using sterile technique, remove masks from packaging and place on working surface of cabinet.
4. Ensure that there is no overlap of adjacent masks, as any unexposed areas will not be sterilized.
5. After transfer, adequately sterilize any external surface that came in contact with the used masks or packaging and destroy the packaging via biological waste.
6. Close the hood and power on the UV light for 20 minutes.
7. After this duration, power off the UV light, open the cabinet, and carefully flip the masks to expose the opposite side, ensuring no overlap of adjacent masks.
8. Close the hood and power on the UV light for 20 minutes.
9. Again, adequately sterilize or dispose of any external surface that comes in contact with the masks.
10. Once the full duration has elapsed, power off the UV light and open the hood.
11. While maintaining sterility of the cabinet, add a tally to each mask indicating the number of UVGI cycles it has experienced and individually place in sterile, sealed packaging.
12. 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 sterilization or decontamination. This method should be implemented *only if* respirators *must be reused*. 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.[6] 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.[4] 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 Lowe et al.[12] In contrast to the eventual degradation of the respirator material, Lindsley et al.[4] found that the straps retained their structural integrity even at high UV doses. This is crucial for maintaining the tight fit of the mask through repeated decontamination cycles.

It should be noted that our recommendation is based on a radiation dose target that is 30 times higher than the dose needed to inactivate 90% of respiratory viruses.[2] If even more dramatic conservation of PPE was required, it would likely be possible to modify this protocol to utilize a lower UV dose. Such a modification may increase the lifespan of each unit of PPE at the cost of potentially incomplete decontamination. More research is needed to determine the number of reuses that could be achieved when decontaminating PPE with a lower UV dose. Though the situation that necessitates the reuse of PPE is far from ideal, using presently-unused BSCs to sanitize N95 respirators has the potential to reduce the current PPE burden by 75%.

Code and Data Availability

All data used in this paper and code written to analyze it are open source and publicly available.[17]

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Author contributions statement

This was a massive team effort with everyone contributing their specific expertise (Fig 4):

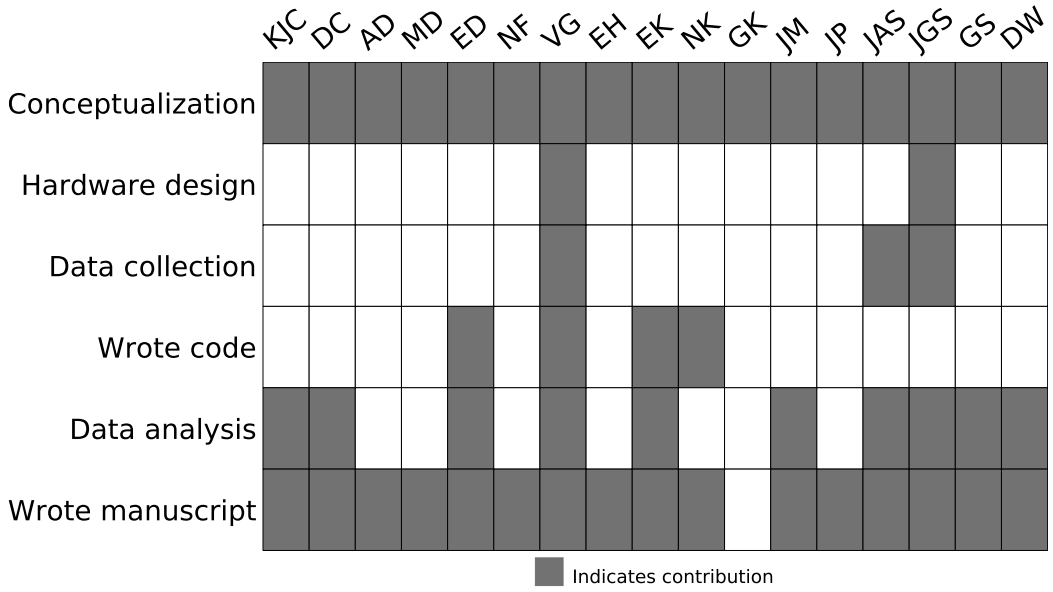

Figure 4. Author contributions

Repeat Disclaimer

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