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LDRD PROJECT NUMBER: 220724
LDRD PROJECT TITLE: CPAP Ventilators Needed for Rapid Response to COVID-19 by Modification of CPAP Equipment
PROJECT TEAM MEMBERS: Ryan Haggerty (PI), Adam Cook, Guild Copeland, Susan Esfahani, Patrick Finnegan, Nate Fuller, Jeff Koplow, Joe Schoeniger, Jason Hinchcliffe, Troy Reese, Steven Wood, Jeff Lynch, Andy Glen, Jesse Cahill, Andres Sanchez, Mike Sinclair, Mike Gallegos, Jim Carney, David Ho, Derrick Higa, William Reinholtz, Marie Arrowsmith

ABSTRACT:

Early on in the COVID-19 pandemic, potential ventilator shortages were a critical issue identified by national health care providers. Capacity modeling at the time suggested patient demand may exceed ventilator supply. Thus, the challenge became finding an urgent interim solution to meet health care needs. Our initial hypothesis was that CPAP technology could be modified to provide similar functionality to a ventilator, relieving demand and allowing physicians to decide which patients need high end machines, ultimately saving lives. In conjunction with medical experts and pulmonologists, we were able to identify three key thrusts associated with this research problem: (1) modification of CPAP technology to allow for O2 input that would be capable of providing ventilation; (2) development of an alarming function that would provide real-time audible alarms to alert medical personnel to critical conditions, which would be used inline with CPAP technology; and (3) a method of sterilizing expiratory air from such a system in order to protect medical personnel from biohazard, since CPAPs vent to the atmosphere. We were unable to realize results for thrust 1 (CPAP modification for O2); we identified potential safety issues associated with utilizing medical grade oxygen with a common CPAP device. In order to characterize and mitigate these issues, we would need to partner closely with a device manufacturer; such a partnership could not be achieved in the timeframe needed for this rapid response work. However, we determined that some medical grade BiPAP devices do not need this modification and that the significant progress on thrusts 2 and 3 would be sufficient to buy down risk of a massive ventilator shortage. Our team built a prototype alarm system that can be utilized with any assistive respiratory device to alert on all key conditions identified by medical personnel (high pressure, low pressure, apnea, loss of power, low battery). Finally, our team made significant progress in the rapid prototyping and demonstration of an inline UV air purifier device. The device is cost efficient and can be manufactured at scale with

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both commercially available and additively manufactured parts. Initial tests with SARS-CoV-2 analog bacteriophage MS2 show 99% efficacy at reducing bioburden. Following a successful demonstration of the prototype device with medical personnel, we were able to obtain follow-on (non-LDRD) funding to provide additional device characterization, validation, and production in order to respond to an immediate regional need.

INTRODUCTION AND EXECUTIVE SUMMARY OF RESULTS:

In the early stages of the COVID-19 pandemic, one of the key issues identified was a lack of ventilator availability for critical patients. Our hypothesis was that CPAP equipment could be modified to provide basic ventilator support to COVID-19 patients, through tailoring CPAP functions to provide variable pressure, handle higher oxygen concentrations, and other relevant functionality based on requirements identified through collaboration with medical collaborators.

Due to the large percentage of the population that has sleep apnea, CPAP equipment is readily available and is produced in large quantities. There are more than 8 million CPAP users in the US and the numbers are increasing by 8-9%/year. These devices provide some of the functionality of ventilators and use pressures in a similar range of 5-40 cm of H2O. We partnered with medical experts and pulmonologists (UNM's School of Medicine, Pulmonary Medicine division) to define requirements for ventilator function that would be therapeutic for a large fraction of COVID-19 patients. We also undertook an initial evaluation of possible modification approaches to obtain performance consistent with the initial medical requirements.

Following this evaluation, we quickly identified several thrust areas to focus on: (1) CPAP modification to provide ventilator support; (2) development of an alarm system that could be utilized in line with a modified CPAP to audibly alert medical personnel in real time of emergent patient conditions; and (3) development of an in line air sterilization system that would decontaminate patient expiratory air when used with a modified CPAP machine or other breathing device.

After examining a CPAP device with the intent of understanding potential usage with medical grade oxygen, we determined that we would need to partner with a device manufacturer to better understand design and safety rationale. We were unable to develop the collaboration needed to make further progress on this thrust. Thus, due to rapid response nature of the project and the R&D potential of the other thrusts, we determined CPAP modification was not feasible. We also determined that this thrust was less critical than thrusts 2 and 3. The majority of CPAP devices do not support O2 on the input, but they often support O2 as a passive addition to the air on the patient side of the device, this does not allow for accurate control of O2 content for the patient,

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but does allow O2 content to be increased. Since this solution already existed, the team deprioritized this part of the project. In addition to this we found that devices that do support O2 on the inlet do exist. They are not as prevalent as common CPAPs, but all hospitals contacted had between 5 and 40 systems that can handle O2 input.

The team did have key wins on thrusts 2 and 3. Examining the CPAP device yielded insight into some features of alarm functionality; namely that while machines are equipped with alarm functions, these typically write to a disk and are used after the fact by a physician to adjust a patient's CPAP regime. Some devices may have audible alarms to alert a user of certain conditions, but there is no standard across brands and devices on alarm functionality. Based on discussions with medical personnel to understand real-time alarm needs (high and low pressure, apnea, loss of power, low battery), the team designed and successfully tested an inline unit that provides audible alarms when critical conditions are met. The device uses a pressure transducer and an Arduino Nano to provide a cost-effective solution that can be coupled with a CPAP or BiPAP system, independent of make and model. There is interest among other laboratories in utilizing the technology in parallel to respiratory aid devices being developed in response to the COVID-19 pandemic.

The most impactful result came out of thrust 3 (development of an inline air sterilization system). The team developed a protype design consisting of a polished aluminum tube that amplifies energy from embedded UV light to decontaminate the exhaled breath by disabling pathogens that pass through. The prototype was developed, built, and tested to ensure sufficient exposure of the entrained pathogens to UV energy. Using a UV light source to kill pathogens, rather than a filter, eliminates the possibility of clogging that is commonly experienced with designs that rely on filters. The unit was tested with the Phillips Respironics V60; however, because the unit does not add any significant flow resistance, it could be retrofitted into other existing respiratory assistance devices. The design utilizes commonly sourced, commercially available, off the shelf parts, with only a few requiring custom manufacturing. To prove the effectiveness of the device, the deployed UV exposure measurements coupled with light-propagation modeling to ensure the design had significant margin over what would be required to kill live coronavirus. Sandia's unique aerosol test facility also was used to ensure the design sufficiently killed live biologicals commonly used to simulate the coronavirus.

The method of air flow sterilization is high-intensity UV irradiation at a wavelength of 254 nm. The sterilization unit uses a commercially available G30 T8 UV germicidal lamp and is treated so as to not generate ozone. The bulb is identical in form factor to a standard T8, 36-inch-long fluorescent light tube. The germicidal lamp is housed inside a 36-inch-long outer tube whose

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interior surface is polished for high UV reflectivity, which enhances the viral kill factor. The efficacy of this sterilization unit was first tested with the surrogate virus phage MS2, which has shown to be more resistant to UV than coronaviruses and killed 99.87% of the MS2 phage.

The electrical components are contained in a separate enclosure; the unit is powered by a standard T8 fluorescent lamp ballast, requiring a standard 120 V outlet. None of the electrical hookups are subject to a hot or humid environment. The electrical enclosure and tube are both grounded. The unit is operated by a rocker switch at the top of the panel. The device is assembled to support maintenance, if needed (the standard lamp operating time is ~8000 hours). The device also features a visual indicator to ensure the device is operating.

The initial prototype was demonstrated to the local medical community, and there was immediate interest in the potential of the technology to help address regional needs. As such, the prototype device, later termed the *Pathogen Management Kit*, received follow-on (non-LDRD) funding that allowed for additional device characterization, maturation, and ultimately production of 100 units.

DETAILED DESCRIPTION OF RESEARCH AND DEVELOPMENT AND METHODOLOGY:

Thrust 1 – CPAP modification for O2

To investigate the feasibility of Thrust 1, we first worked with medical personnel to understand basic both basic ventilator operation and CPAP operation. A key concern for COVID-19 patients is keeping them oxygenated. When a ventilator is attached to a patient, oxygen is set for a particular concentration and adjusted based on patient response until the desired level is reached. Typical oxygen supply lines operate at 50 psi and are either 50% oxygen or 100% oxygen; normal ventilators operate at 100% O2, which is lowered as the patient improves.

We surveyed various CPAP and BiPAP units as potential candidates for modification, and focused efforts on BiPAP (rather than CPAP) due to the variable pressures for inhalation and exhalation. Online documentation of various models indicates most designs utilize a motor to drive a fan at different speeds for inhalation and exhalation. Older models may utilize a square wave pressure, which is harder on the patient. Newer models use a pulse waveform, where pressure ramps up and down and avoids this “hammer effect” of the square wave. While some machines have oxygen inputs, it is atypical for them to be compatible with the 50 psi supply lines used in hospitals.

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Our team had access to a ResMed AirCurve 10, which was disassembled and examined to better understand a potential path forward for modification, with respect to oxygen utilization. One possibility was to run oxygen through the device, but safety and practicality concerns arose with this approach (is the motor oxygen safe under both 100% or lower concentration conditions, and if not, would it be feasible to replace with an oxygen safe motor). Another possibility would be to provide O2 after the device, but this limits the % of O2 we could work with in such a mode. A third possibility was to filter the air prior to injection into the machine (versus working with pure oxygen); machines contain a built-in filter, but efficacy information was unknown.

In order to better understand the logistics of these potential approaches, we determined we would need to work more closely with a BiPAP manufacturer. It would be critical to review drawings and speak with their engineers on specific functions and limitations of the machines. Additionally, we determined we would also need to access medical grade O2 to test potential approaches and assess safety issues. We were not able to establish the needed industry connections to facilitate further development of the BiPAP modification approach in a timeframe that would be meaningful in a rapid response scenario.

Thrust 2. System independent alarms

As we consider the use of a CPAP or BiPAP unit in a hospital setting as an emergency-use respiratory device, a key consideration is how the unit will interact with both the patient, as well as the hospital staff. Most consumer units capture the data the hospital staff find valuable, but they do not do it in a format that is real time as it pertains to the patient. One example are the Phillips brand machines, which have audible alarms for functions such as Low Minute Vent, Apnea, Patient Disconnect, Device Inoperative, Inlet Blocked (check Filter), Device Inoperative, Loss of Power, Low Pressure, High Pressure, Low Motor Speed. This is useful data, but the alarm information is not necessarily exported in real time; it is stored as a log the user can download after the fact to examine. This must be done in cooperation with the manufacturer, as they have the sensors built in with wireless communication. Even if the data could be downloaded by the end user in real time, it addresses only one brand. The ideal state would be to utilize as many CPAP/BIPAP units as possible as respiratory aids. This is the motivation to develop a system that operates independently of the CPAP/BIPAP and communicates the necessary alarms to the hospital staff.

To address this problem, we considered several potential solutions:

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- Solution 1: Use a simple mechanical pressure switch, which would monitor only for high pressure (>40hPa). While this is a possibility it only solves one condition and usefulness is rather limited.

- Solution 2: Use a USB Pressure Sensor. This can connect to laptop to monitor continuously every 10 milliseconds and could be used to detect clog or disconnect. While this is a better solution, it is cumbersome as it now requires the use of a laptop and does not necessarily address all the alarms.

- Solution 3: Use a COTS pressure transducer, and code an Arduino Nano to look for the alarm states and alarm as necessary. There is some concern that the extra weight of such a sensor at the tube could interfere with the patient. We planned to mitigate this by having the sensor and associated processor in a unit that would sit next to the CPAP/BIPAP unit and only have a small tube connected to a standard hospital BIPAP O2 adaptor to sample pressure. The pressure transducer selected for initial development was a MPX10. This was later switched to a MPXV7002, as it has a built-in amplification circuit. This a popular component used by drone pilots to measure speed and as such there is opensource code available to get us going quickly.

We talked with hospital staff about what alarms would be most useful, as well as ranges for those alarms. Three critical alarms were identified: high pressure alarm at >40hPa, low pressure alarm <6hPa, and an apnea alarm. While consumer units typically never see pressures at 40hPa, leaving this as a higher range was still desired by hospital staff to indicate a machine malfunction. Additionally, hospital staff generally prefer units of cmH20 (versus hPA), so we adapted our process to utilize units familiar to hospital staff. In order to better understand pressure behavior during the code development phase, we quickly realized we needed real data to help understand and bound thresholds. While waiting for permission from the human studies board, we attempted to create breathing data with an artificial breathing lung (basically a balloon connected to a CPAP unit). While this useful to first order, it was not a substitute for real data. We were able to obtain real CPAP data from a long-term user, decode the data, plot it, and utilize it to help refine our code. The raw data is very noisy, so a library was imported to smooth the breathing data, making it is easier to set thresholds for alarms (Figure 1).

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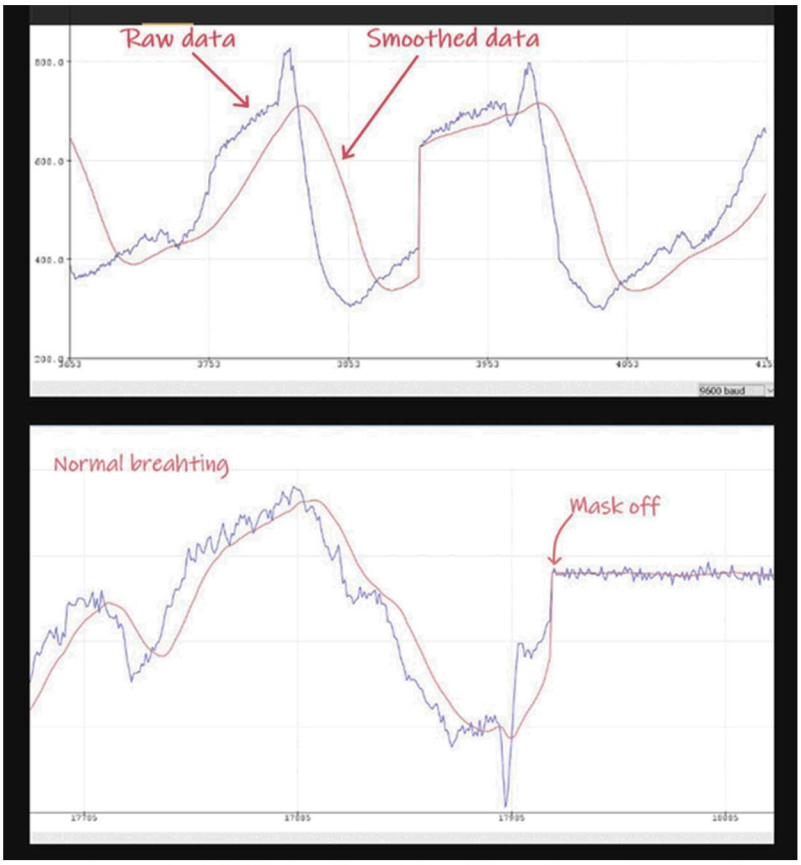

Figure 1. Example of raw and smoothed CPAP data illustrating different breathing scenarios (normal, mask off).

We were able to develop alarms for the high- and low-pressure points, as well as loss of power, and low battery. The alarm for apnea presented additional challenges to code. We decided to use 250 samples of the normal breathing oscillations and calculate the Max and Min for those points. If that delta over 250 samples (~10 second window) falls below 200 pascals, the APNEA alarm will be set. The system will continue to look at the high and low respiratory cycles and update average. This allows the unit to self-adjust for sleeping/wake times.

When the system enters alarm mode for any of the conditions described above, the alarm is continuous. When responding to the alarm, medical personnel will press a button to disable the alarm for 5 minutes to address the issue. After that 5 minutes, the alarm function is reset and reenabled. During an alarm for a power disconnect, the alarm behaves more like smoke detector,

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beeping intermittently until power is reconnected. Additionally, the alarm displays a low battery icon (no audible alarm) when the battery runs low.

While the code development was in progress, the team switched the pressure transducer to a MPXV5010GC7U-ND, as it gives us a higher overhead, through hole design, and the ability to hand manufacture. This enabled the team to continue to solve the math apnea problem, while we began board development. The PCB layout includes a provision to use a MPX5010 (primary) or a MPXV7002 (alternate part) transducer. We also made use of Adafruit power boost 1000 as it solves the charging, discharging and power requirements of our design and is easily interfaced to the NANO.

Thrust 3 – Inline air sterilization system.
Normal ventilators collect the expiratory air and run it through a HEPA filter, but other systems lack this capability, and may typically only have one line that vents to atmosphere. While such non-invasive ventilation options have shown promise in treating candidate patients and are sometimes preferred over intubation, there have been limited solutions to address the contamination concerns. For example, helmet solutions exist but are of in limited use in the United States (U.S). In-line filters can cause pressure changes over time and get clogged. In order to protect medical personnel from aerosolized pathogens present in a patients expiratory breathing cycle, it is critical that respiratory aid devices be equipped with air treatment system.

To address this challenge, we developed a prototype in-line Ultraviolet (UV) sanitization system. UV radiation kills viruses by chemically modifying their genetic material, DNA and RNA. The most effective wavelength for inactivation is around 260 nanometers (Lytle and Sagripanti, 2005), and falls in the UVC range. The efficacy of germicidal UV depends on the length of time a microorganism is exposed to UV, the intensity and wavelength of the UV radiation, the presence of particles that can protect the microorganisms from UV, and a microorganism's ability to withstand UV during its exposure.

The prototype system consists of a polished aluminum tube that amplifies energy from embedded UV light to decontaminate the exhaled breath by disabling pathogens that pass through. The design philosophy was to use only commonly sourced parts with no supply chain issues. Most of the parts are commercially available, off the shelf (COTS), with only a few requiring custom manufacturing.

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Design rationale

Simple analytical calculations were performed to guide the design of the UV disinfection system. The starting point for the calculations is the total dose of 254 nm radiation required to achieve a desired reduction in virus particles. In consultation with SME Jim Carney, a dose of 50 mJ/cm^2 was chosen based upon literature results, which show that this dose is sufficient to reduce the population of MS2 macrophages by 2-3 orders of magnitude. Coronaviruses are known to be much more sensitive to UV radiation and easier to destroy.

Next, the 254 nm power radiated by the bulb was calculated as a function of the radial distance from the center of the bulb. For radial distances not too large compared to the radius of the bulb, the power was assumed to be inversely proportional to the distance. In this manner, the power at the inner wall of the aluminum tube could be calculated as a function of the tube diameter. To account for multiple reflections of the UV light within the tube, the power at the inner diameter of the aluminum tube was multiplied by an experimentally determined parameter called the "reflectivity enhancement factor".

This factor was determined by measuring the power emanating from a 3 mm hole in the sidewall at the midpoint of a prototype tube design using a calibrated optical power meter (the sidewall in the vicinity of the hole was thinned to 1 mm to allow a wide range of incidence angles to pass through the hole). A crude detector calibration was employed to account for the fact that low levels of light at other wavelengths are produced by the bulb. The ratio of power emanating multiplied by 1.9 (to account for the reflection from the sidewall) to the calculated power was taken as the reflectivity enhancement factor. Using the power corrected for the reflectivity enhancement, along with the required UV dose, the residence time required for virus particles within the tube can be calculated.

In turn, the residence time, along with the bulb length determines the maximum velocity allowable for the air flow through the tube. Next, the flow velocity in combination with the open cross-sectional area (aluminum tube inner cross-sectional area minus the bulb cross-sectional area) determines the volumetric flow. A simple Python script was written to perform these calculations and allowed for factors such as the aluminum tube diameter, the tube length, and the required flow rate to be varied. This calculation procedure is over simplified and can only be relied upon for order-of-magnitude estimates. It could be greatly improved by: (1) using optical ray tracing to calculate the 3-dimensional light field distribution within the tube; and (2) using computational fluid dynamics to calculate the actual gas flow field within the tube.

Thinning the sidewall of the production tubes was not possible, and a tapped ¼ inch diameter hole was desired for a visual indicator of tube operation. To provide a rough calibration between the power from the tapped hole (without a thinned sidewall) and the power measured from a hole

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in a region of thinned sidewall, we used our prototype system to measure the power from a 3 mm hole with thinned sidewall along with the power from a ¼ inch tapped hole in a region where the sidewall was not thinned. The UV light emanating from the ¼ inch tapped hole will be used to record the output of all the units produced.

In addition to understanding UV parameters, the team worked with material scientists to understand constraints related to operating in an oxygenated environment, with respect to potential additively manufactured device components. Six constituent materials were provided for thermal analysis under oxygen enriched environment, to determine the temperature at which each material begins to break down.

Tests were carried out in an STA 449 F3 Jupiter thermal analyzer (Netzsch Instruments, Selb, Germany). Specimens of between 10 and 30 mg were placed into a clean alumina crucible and loaded into the thermal analyzer. A gas flow of 95 sccm pure oxygen and 5 sccm pure argon was established through the instrument's sample chamber (= 95% oxygen atmosphere). Once the sample mass had stabilized, the sample was heated at 10 °C/min to 600 °C while continuously monitoring sample mass (thermogravimetric analysis), while maintaining the same gas flow. Each sample was analyzed one time to achieve a quick turnaround of results.

Efficacy Testing Approach

To test the efficacy of the prototype device, we utilized the live virus, phage MS2, which serves as a surrogate for the SARS-CoV-2. There is precedent that coronaviruses are more sensitive to UV than MS2. Turgeon et al (2014) showed that phage MS2 was the most robust among five phages tested, as it could be detected by plaque assays at similar levels. Additionally, phage MS2 is seven to ten times more resistant to aerosolization, sampling, and UV light than a coronavirus (Walker and Gawngpyo, 2007).

Necessary safety protocols were completed to facilitate testing and validation of the device's operation. Various device sizes were tested with live virus to understand any effects of geometry, and to gather a statistical sample. The devices were tested using range of flow rates, to simulate all possibilities of ventilator operation. Additionally, an ozone photometer was placed on the unit to confirm that no ozone was generated during operation.

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RESULTS AND DISCUSSION:

Thrust 1 – CPAP modification. As described previously, due to time constraints, we decided to discontinue further efforts on this thrust. Fully developing this concept would require collaboration with a BiPAP manufacturer in order to better understand some of the limitations and safety concerns of current models.

Thrust 2 – System independent alarms.
We have developed a system that alarms to the following conditions:

Alarm Table			
Alarm	Set Point	Auditable Indication	Reset Function
Low Pressure	x<6 cmH2O/3sec	Constant	Acknowledge button
High Pressure	x>30 cmH2O/3sec	Constant	Acknowledge button
APNEA	No breath in 10sec	Constant	Acknowledge button
Loss of AC power	No 5V external power	Intermittent	Self-resets when power is re applied
Low Battery	Battery less than 3.5V	Constant	Acknowledge button

Figure 2 shows the latest PCB layout for the system:

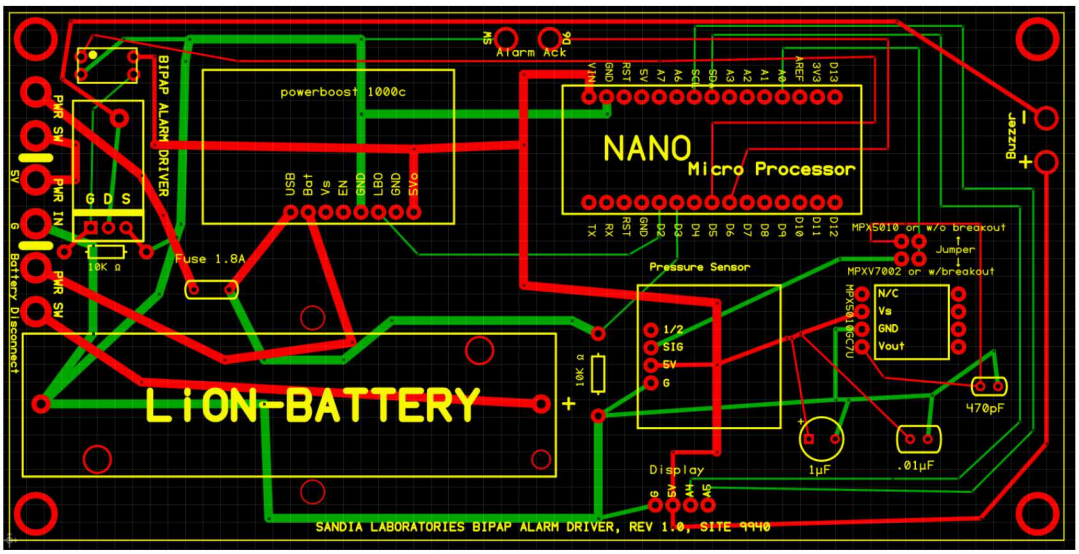

Figure 2. PCB layout for alarm system.

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Thrust 3 – Inline air sterilization system

Design validation

The modeling efforts and oxygen compatibility results informed the device design, specifically the sizes of the lamps and tubing; the longer and wider the device, the more time the virus is exposed to the UV. The device was designed using a combination of COTS and advanced manufactured parts, which were selected both on safety basis (operation in oxygenated environment), enhanced efficacy (ability to kill the virus), and commercial availability and ability to rapidly scale. Standard aluminum tubing was selected, which is then polished on its inner surface. Measured data on prototype devices suggest that polished aluminum improves the UV intensity by a factor of five, which would enhance the kill rate, as shown in the modeling results in Figure 3. Numerous combinations of diameter and length were tested, determining that the 4″ x 36″ configuration would provide the necessary geometry to achieve the kill rate.

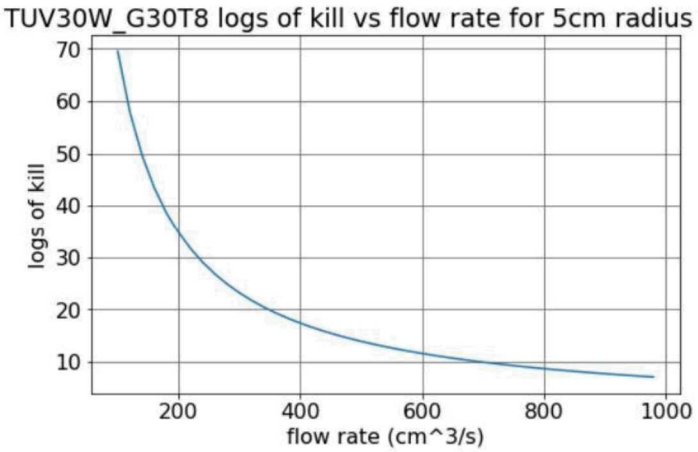

Figure 3. Models of logs of kill as a function of flow rate for a 5 cm radius polished aluminum tube (corresponds to 4” diameter) assuming a 50 mJ/cm2 of 254 nm radiation.

The results of thermogravimetric analysis (material compatibility tests) are shown in Figure 4 and Figure 5. As can be seen in Figure A-1, PLA does not lose mass until above 300 °C, while the two samples based on clear V4 show a minor gradual mass loss between 100 and 250 °C (probably solvent loss), followed by more rapid mass loss (decomposition) above 275 °C.

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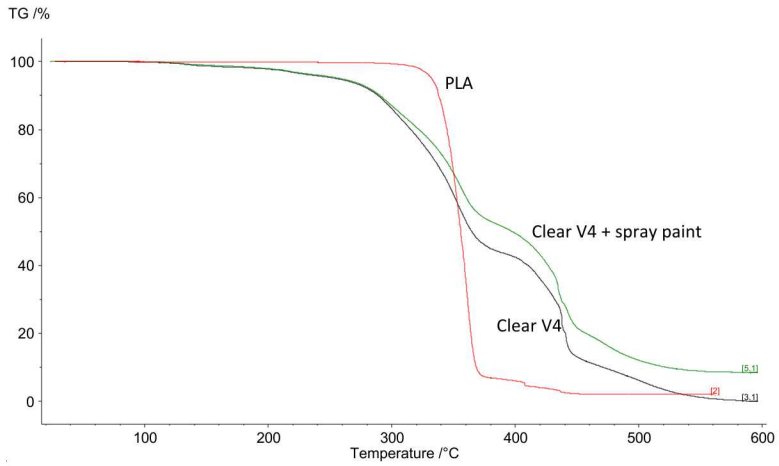

Figure 4. Thermogravimetric analysis of oxygen compatibility of PLA, Clear V4, and Clear V4 with spray paint. Ramp rate: 10 °C/min; gas flow: 100 sccm 95% O2, balance argon.

Both polycarbonate wire and Ultem 1010 wire showed excellent thermal stability under 95% oxygen, with mass losses beginning at ca. 390 °C and 490 °C, respectively (Figure 5). Vero, on the other hand exhibited a gradual mass loss below about 250 °C, similar to the clear V4 materials. Mass loss rate increased for Vero at around 270 °C, indicating decomposition.

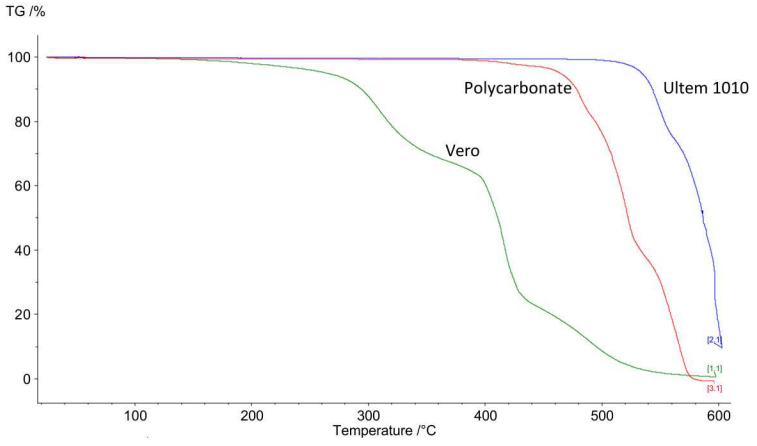

Figure 5. Thermogravimetric analysis of oxygen compatibility of Vero, Polycarbonate, and Ultem 1010. Ramp rate: 10 °C/min; gas flow: 100 sccm 95% O2, balance argon.

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In summary, all the materials studied appeared to be stable under 95% oxygen up to at least 100 °C. The minor mass losses seen for Vero and clear V4 below 250 °C are tentatively assigned to solvent loss, thereafter they begin to decompose around 270 °C. PLA, polycarbonate, and Ultem 1010 proved stable to > 300 °C.

Efficacy testing

The prototype device was experimentally tested at the Sandia National Laboratories Aerosol Complex on April 10 and April 11, 2020.

MS2 bacteriophage RNA concentrations were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR). RNA was isolated from MS2 samples using a Zymo ZR Viral DNA/RNA kit. Reverse transcriptase and PCR were accomplished in 25 µL reactions with TaqMan Fast Virus 1-step Master Mix. Detection targeted an assembly protein gene, with reaction conditions and concentrations described by O'Connell, et al. (2006). Primers and probe sequences from O'Connell et al. are shown below:

MS2 Assembly Target	Forward	5' to 3'	GTCGCGGTAATTGGCGC
	Reverse	5' to 3'	GGCCACGTGTTTTGATCGA
	Probe	5' to 3'	**56-FAM**-AGGCGCTCCGCTACCTTGCCCT-**36-TAMSp**

All samples were processed in triplicate, using a Bio-Rad CFX Connect, with no template controls and representative samples from the MS-2 stock-based standard curve, to ensure consistency of reverse transcriptase and PCR activation. Figure 6 represents the positive identification of MS2 within the target assay.

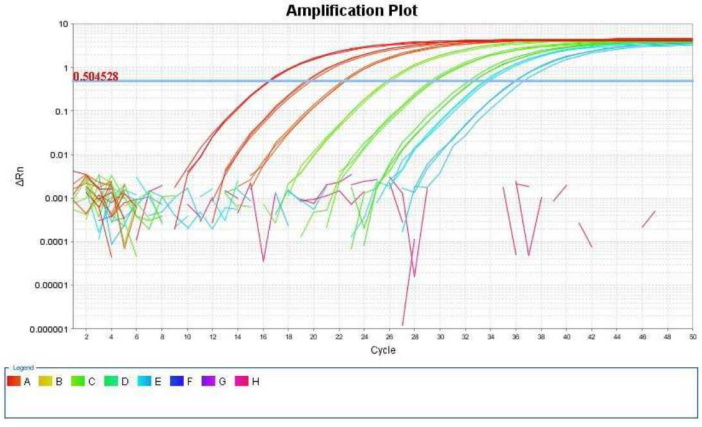

Figure 6. qRT-PCR standard curve.

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A high titer MS2 lysate (1.3 x10^{10} PFU/mL) was prepared by double agar layer method. Briefly 1 x10^{5} PFU of MS2 was mixed with 100 µL of an overnight culture of NEB Turbo and incubated at room temperature for 10 min. This mixture was added to with 4 mL of molten top agar (0.7% Difco biological agar in LB broth, supplemented with 5 mM $CaCl_2$ and 10 mM $MgSO_4$) and immediately poured on a pre-warmed LB (1.5% Difco agar) plate, forming a bacterial lawn. Plates were incubated at 37 °C. The next day, the top layer from ten replicate plates was harvested in 30 mL of lambda dil (25 mM Tris-HCL pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$, 0.01% gelatin), centrifuged to pellet cell debris and agar, and the supernatant was sterilized using a 0.2 µm filter.

The experimental setup is shown in Figure 7. The setup uses a syringe pump and Sono-Tek nebulizer to generate a constant output of MS2 aerosol into a large mixing chamber. The air stream in this chamber is then directed to a manifold upstream of the Product. At the upstream manifold are sample lines going to an upstream impinger, to a TSI Aerodynamic Particle Sizer (APS) and to the Product. After the Product is another manifold with sample lines going to the downstream impinger, the APS and an ozone photometer (2B Technologies, Ozone Monitor 106-L), any additional air is exhausted through a HEPA filter. The entire setup was installed inside a secondary containment bioaerosol chamber.

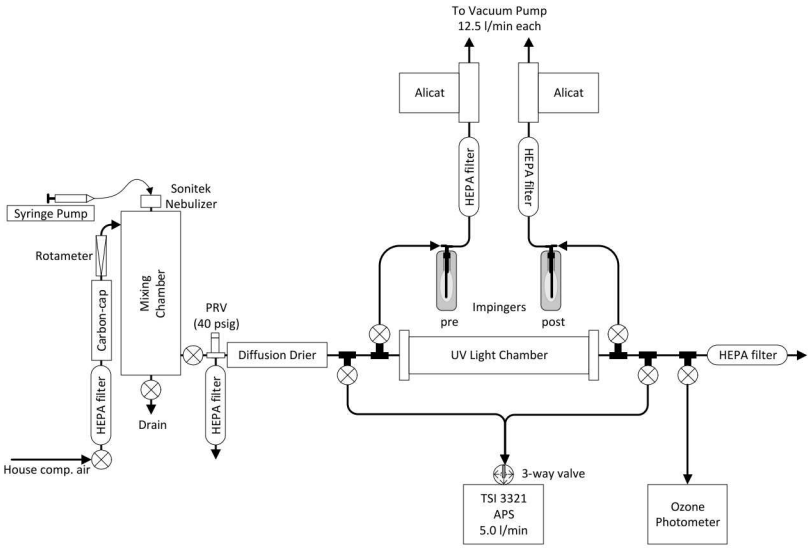

Figure 7. Experimental Setup

During the experiment the impingers were initially closed off from the flow system, allowing the system to achieve steady state conditions. Aerosol size distributions were measured by the APS

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unit at both the upstream (Pre) and downstream (Post) locations. Only a single APS was used and switched between the pre and post-Product locations to reduce uncertainty in measurement differences. Once the system was in steady state conditions, the valves to the impingers were opened and they sampled aerosol-laden air for 5 minutes at a flow rate of 12.5 L/min. The flowrate through the prototype device while the impingers were sampling was 30 L/min during these tests.

Once the test was complete, the impingers were disconnected from the test system and the openings covered in parafilm. A serological pipette was used to transfer the sample from the impinger to a 50 mL sterile centrifuge tube and the final volume was recorded. The samples were stored in the refrigerator until they were ready for plaque assay processing.

Phages were quantified using the spot titer method. Briefly, 100 µL of an overnight culture of NEB Turbo was added to 4 mL of molten top agar and immediately poured on a pre-warmed LB plate. Phages were diluted in lambda dil and ten microliters of the phage suspension was spotted on the lawn in a ten-fold series ranging from undiluted to the 10^{-8} dilution. Plates were incubated at 37 °C. Phages were quantified from plaques the next day.

Due to the urgent need, the prototype device was tested only under one flow rate (worst case) scenario (30 L/min). Three replicate tests were conducted along with one positive blank (where MS2 aerosol was present but the Product was powered down). The impingers were prepared with 20 ml of Phosphate Buffered Saline with Tween (PBST) and 50 µL of Anti-Foam B. All samples were collected for 5 minutes at a flow rate of 12.5 L/min. The table below shows the sample volumes and the absolute number of neutralized MS2 Plaque Forming Units (PFU) in both the impinger sample and normalized by the air flow rate. Additionally, the neutralization efficiency/“kill” efficiency is included as a percentage of the total number of PFU.

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Sample	Sample Volume		Titer Concentration (Plaque Forming Units /mL)	Neutralized Particles		Neutralization Efficiency (%)
	Initial (mL)	End (mL)		($PFU/mL_{impinger}$)	(PFU/L_{air})	
Replicate 1 Up *	20	20	1.90E+07	1.90E+07	6.07E+06	99.78%
Replicate 1 Down*	20	20	4.20E+04			
Replicate 2 Up*	20	20	1.50E+07	1.50E+07	4.79E+06	99.89%
Replicate2 Down*	20	20	1.60E+04			
Replicate3 Up*	20	20	1.20E+07	1.20E+07	3.84E+06	99.94%
Replicate 3 Down*	20	20	7.00E+03			
Blank Up	20	14.8	1.20E+07	0.00E+00	0.00E+00	0.00%
Blank Down	20	16.7	1.20E+07			
			Average	1.53E+07	4.90E+06	99.87%

* Added 50uL of Anti-Foam B

The aerosol size distribution was measured both pre and post-device during the tests. Parameters describing the aerosol size distributions are shown below. The average aerosol concentration measured by the APS during the tests were between 150 and 220 $\#/cm^{-3}$ and had a mode diameter between 1.5 and 2.1 µm. An example size distribution is shown in Figure 8, for the Replicate #2 test. As the figure shows, the distribution is very similar between the upstream and downstream of the Product.

APS Data			
Sample	Mode Diameter (µm)	Geo. Std. Dev.	Total Concentration ($\#/cm^{-3}$)
Replicate 1 Up	1.59	1.47	221.21
Replicate 1 Down	1.52	1.43	183.55
Replicate 2 Up	2.13	1.39	201.83
Replicate2 Down	2.13	1.33	198.14
Replicate3 Up	2.13	1.40	159.77
Replicate 3 Down	2.13	1.37	138.90
Blank Up	1.76	1.39	213.51
Blank Down	1.70	1.40	157.51

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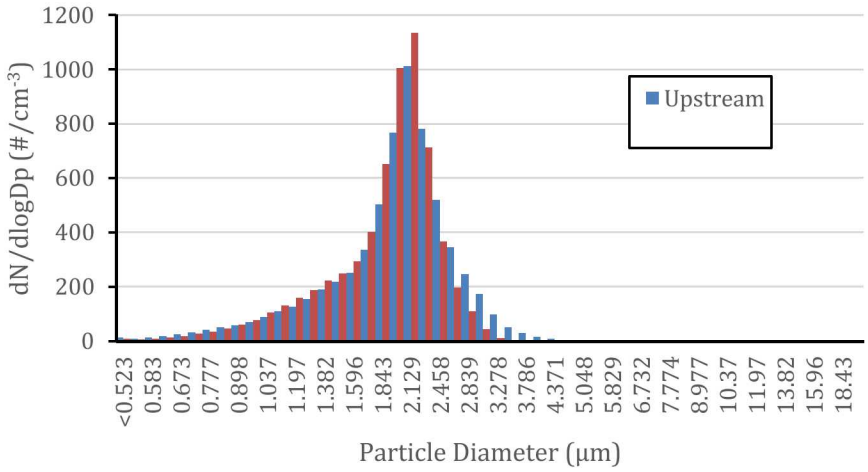

Figure 8. Replicate 2 Size Distribution

Ozone was measured on the downstream side of the device during the MS2 neutralization tests. The data show an average concentrate of 1.8ppb during both MS2 and clean air flow through the device. This value is well below the 50 ppb (0.050 ppm) threshold.

ANTICIPATED OUTCOMES AND IMPACTS:

Thrust 2 – System independent alarms. The simple system we have devised is simple and inexpensive to manufacture and get easily be used in line with respiratory aid devices, such as CPAP or BiPAP machines. It provides critical functionality in a hospital setting to alert medical personnel to key patient conditions or machine malfunctions. There is currently interest at other laboratories in utilizing the technology in conjunction with other concepts.

Thrust 3 - Inline air sterilization system. The prototype device, built with parts that are easy and cost effective to procure and assemble, can be used with non-invasive respiratory devices to provide treatment while keeping medical personnel safe. Evolving research during the time of this project indicated that some portion of COVID-19 patients may experience better outcomes with non-invasive respiratory treatment. Thus, our system met an additional emerging need to broaden the range of options available to medical providers (e.g., using CPAPs as intended in serial with the air sterilization device to protect medical personnel treating the infected patient). Additional device characterization, testing, and production was picked up with non-LDRD follow-on funding, and 100 units were ultimately produced and made available to regional

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medical providers. See the new release: https://share-ng.sandia.gov/news/resources/news_releases/ventilator_kits/ for more information about the impact of the work.

CONCLUSION:

The initial focus on this LDRD project was to test the feasibility of quickly converting commercially available CPAP/BiPAP machines into ventilators to address an anticipated demand due to COVID-19. While we ultimately were not able to realize that aspect of the research, our partnership with the medical community helped to further refine the problem into additional, related components: if a CPAP could be modified, a lack of an audible alarm system for critical conditions would make it difficult to effectively utilize; if a CPAP could be modified or used for treatment, medical personnel would be at risk of exposure to biohazard due to the CPAP design of venting to the atmosphere. Our team was able to address these components by developing a system independent alarm that could be easily coupled with respiratory aid devices. Other laboratories are interested in exploiting this technology with ventilator systems they have developed. Additionally, we developed a prototype device that sterilizes the exhaled breath from a respiratory assistance machine by exposing the breath to UV light that kills pathogens, including COVID-19, before the exhaled breath is vented into the room. UV disinfection is an established method using short wavelength ultraviolet light to kill or inactivate microorganisms and viruses by destroying nucleic acids and disrupting their DNA. Follow-on funding (non-LDRD) allowed for additional maturation of the prototype technology, and 100 units were produced to be deployed regionally to meet emerging needs.

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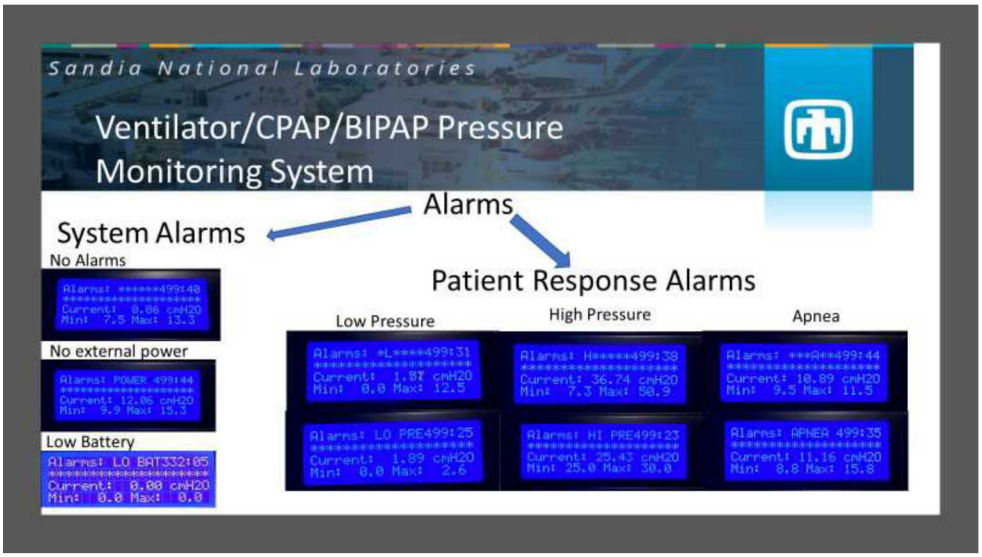
ADDENDUM:



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REFERENCES:

Beck S.E., Rodriguez R.A., Hawkins M.A., Hargy T.M., Larason T.C., Lindena K.G., "Comparison of UV-Induced Inactivation and RNA Damage in MS2 Phage across the Germicidal UV Spectrum", Applied and Environmental Microbiology 82, 1468 (2016).

Covidien, Product Requirements Document PB60-520, Document Number 10035480 (proprietary)

Lytle CD, Sagripanti JL. Predicted inactivation of viruses of relevance to biodefense by solar radiation. *J Virol*. 2005;79(22):14244–14252. doi:10.1128/JVI.79.22.14244-14252.2005

Mt. Sanai, *Mount Sinai Turns Hundreds of Machines for Sleep Apnea into Hospital Ventilators, Shares Instructions Worldwide*, https://inside.mountsinai.org/blog/mount-sinai-turns-h

Praxair Materials Compatibility, https://www.ualberta.ca/chemistry/media-library/safety/gasmaterialcompatability.pdf

Turgeon N, Toulouse MJ, Martel B, Moineau S, Duchaine C. Comparison of five bacteriophages as models for viral aerosol studies. *Appl Environ Microbiol*. 2014;80(14):4242–4250. doi:10.1128/AEM.00767-14

Verreault, Daniel & Moineau, Sylvain & Duchaine, Caroline. (2008). Methods for Sampling of Airborne Viruses. Microbiology and molecular biology reviews: MMBR. 72. 413-44. 10.1128/MMBR.00002-08.

Walker, Chris & Ko, Gwangpyo. (2007). Effect of Ultraviolet Germicidal Irradiation on Viral Aerosols. Environmental science & technology. 41. 5460-5. 10.1021/es070056u.

Real-Time Fluorogenic Reverse Transcription-PCR Assays for Detection of Bacteriophage MS2. Kevin P. O'Connell et al., Applied and Environmental Microbiology Jan 2006, 72 (1) 478-483; DOI: 10.1128/AEM.72.1.478-483.2006

Adams, Mark Hancock. "Bacteriophages." *Bacteriophages.* (1959).

Kropinski, Andrew M., Amanda Mazzocco, Thomas E. Waddell, Erika Lingohr, and Roger P. Johnson. "Enumeration of bacteriophages by double agar overlay plaque assay." In Bacteriophages, pp. 69-76. Humana Press, 2009.

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