Title: A practical PPE decontamination method using warm air and ambient humidity.

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Abstract: Personal protective equipment (PPE) remains in short supply. Current decontamination methods are complex, slow, expensive and particularly ill-suited for low to middle income nations where the need is greatest. We propose a low temperature, ambient humidity decontamination method (WASP-D) based on the thirty minute or less half-life of Sars-CoV-2 (and other common pathogens) at temperatures above 45°C, combined with the observation that most PPE are designed to be safely transported and stored at temperatures below 50°C. Decontamination at 12 hours, 46°C (115°F) and ambient humidity should consistently reduce SARS-CoV-2 viral load by a factor of 10^{-6}, without negatively affecting PPE materials or performance.

Introduction:
In the midst of a pandemic, Personal Protective Equipment (PPE) are a critical line of first defense. Even before vaccines and treatments are developed, PPE limits the spread of disease and protects the lives of vital healthcare workers. Unfortunately, PPE manufacturing capacity and stockpiles persistently lag demand, resulting in needless deaths and severe economic disruption. Despite best efforts to expand capacity, this PPE access gap continues in developed economies with strong healthcare systems and is exacerbated in low to middle income nations (LMIN)[1].

One potential stop-gap measure is to safely and effectively decontaminate disposable PPE for re-use. A small cohort of methods have been evaluated and approved for use by the CDC and WHO for N95 masks (knowledge here is rapidly evolving- see N95Decon.org or the CDC[2] for current information). These include UV-C irradiation, vaporized hydrogen peroxide, dry and moist heat[3].

While viable, these methods fail to work uniformly on all brands of PPE[4], require costly electronics/pumps /renewables and maintenance, or do not scale to high capacity. For example, UV-C is a broad-spectrum antimicrobial, but cannot penetrate into the shadows of folded PPE which may harbor viable pathogens. Some UV systems produce ozone which breaks down[5] plastic surfaces. Chemical treatment such as peroxide vapor is both toxic and requires specialized equipment and staff to operate at large scale. To avoid inadvertent high-temperature thermal degradation from exposed 1500°F electric-heating elements, moist heat (60% RH and 165°F/74°C) ovens must be specially designed to block infra-red radiation[6]. High

[1] https://www.ebmt.org/low-middle-income-country-lmic-membership
[2] https://www.cdc.gov/coronavirus/2019-ncov/hcp/ppe-strategy/decontamination-reuse-respirators.html
[3] https://www.fda.gov/media/138284/download
[4] https://www.battelle.org/inb/battelle-ccds-for-covid19-satellite-locations
[5] https://en.wikipedia.org/wiki/Ozone_cracking
[6] https://www.n95decon.org/publications#heat

heat also softens elastomeric straps, which may not return to their original taut lengths, or may reduce the material's ultimate tear strength.

N95 masks, in particular, contain an inner layer that is electrostatically charged to enhance particle collection efficiency without impacting breathability. This layer is easily neutralized by many wet sanitizers, such as soap and water or autoclaving, making re-use impossible. Other PPE, such as gowns or face shields, can be decontaminated in liquid disinfectants, though may be damaged by physical impact and the process itself is highly labor intensive.

Our goal is to devise and test an accessible decontamination protocol suited for LMIN[7]. We are seeking a decontamination, not a sterilization, methodology. The key metric is reducing viral burden and associated clinic or hospital pathogens to manageable levels. We recognize that some pathogens, e.g. mesophiles such as Listeria[8] and methicillin-resistant Staphylococcus aureus (MRSA)[9], have an active growth rate upper temperature bound that just extends into the WASP-D range. And that no lab test, including this study and associated literature studies, can eliminate the possibility of post-WASP-D decontamination re-contamination. But we also note the world is filled with pathogens- every breath[10] we take inhales e-coli, clostridium botulinum and various actinobacteria, along with air pollution and other toxic fumes. Reduction from harm, not elimination of all possible risks, is the goal in a pandemic.

Such a protocol would ideally meet the following criteria:

1. Achieve at least 10^6 reduction in common disease pathogens.
2. Inexpensive.
3. Work on all brands and all types of PPE without damage.
4. Tolerant of intermittent power outages.
5. Constructed locally.
6. Minimize training to operate.
7. Fail-soft.

Simply "waiting" at room temperature has been demonstrated to reduce SARS-CoV-2 by three orders of magnitude over seven days[11]. However, according to the CDC, six orders of magnitude reduction is typically required for safe re-use[12]. Characteristically, pathogens exhibit a thermally activated degradation rate, but the literature contains few reliable measurements of warm temperature viral inhibition. Pathogen half-life depends on a number of external factors, including temperature, humidity, exposure to light (particularly UV), encasing media (e.g. saliva,

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[7] https://www.cdc.gov/coronavirus/2019-ncov/hcp/non-us-settings/emergency-considerations-ppe.html
[8] http://textbookofbacteriology.net/Listeria.html
[9] http://textbookofbacteriology.net/staph.html
[10] *Urban aerosols harbor diverse and dynamic bacterial populations*, Eoin L. Brodie, Todd Z. DeSantis, Jordan P. Moberg Parker, Ingrid X.Zubietta, Yvette M. Piceno, Gary L. Andersen, Proceedings of the National Academy of Sciences Jan 2007, 104 (1) 299-304; DOI:10.1073/pnas.0608255104
[11] https://www.n95decon.org/implementation#time
[12] https://www.fda.gov/media/138362/download

mucous, water, …) and substrate[13] (metal, plastic, mesh, air ..). While Sars-CoV-2 research continues to advance, literature results to-date indicate a factor of four or more variation[14] in half-life depending on these external factors. Thus, any practical system must err on the side of caution regarding temperature and humidity.

The purpose of our study is to determine if low-temperature warming of viruses can achieve a six-order of magnitude viral load reduction. Here we test the ability of low-temperature warming to inactivate two surrogate viruses – bovine coronavirus, a beta coronavirus, like SARS-CoV-2 (enveloped) that infects cattle, and MS-2, a small, non-enveloped virus that infects E.coli.

By far the simplest solution would be a combination of time and warm heat at ambient humidity- **W**arm **A**nd **S**low **P**athogen-**D**econtamination, or WASP-D. In WASP-D, PPE are placed in a large closed room or container. Warm heat (43°C-50°C=110°F-122°F, nominally 46°C as a safety factor) and ambient air ventilation are introduced for 12 hours, following the protocol at https://www.protocols.io/view/wasp-d-field-protocol-bkcskswe . After 12 hours, the amount of infectious virus is reduced by a factor of 10^6.

Effective, universal, low-tech, scalable and no consumables.

WASP-D Parameter Choices:

There are three key parameters in this study. First, the choice of warm heat at 43°C-50°C. Second, a relative humidity characteristic of affected communities. And third, a pathogen reduction of 10^6 or greater. Decontamination cycle time is a dependent variable necessary to achieve the above objectives.

Warm temperatures: Most PPE are manufactured from plastic. For example, melt spun polypropylene for the N95 electrostatic filter or high-density polyethylene in a Tyvek body suit. Cellulosic padding, natural or thermoplastic elastomers and small metal snaps may be part of the design. In addition, proprietary hydrophilic coatings to repel blood or bodily fluids, anti-microbials and a host of unknown and transient features may be present. Each material will experience its own specific thermally activated failure mechanism and degradation pathways.

Testing every brand of PPE is impractical. However, almost all PPE are intended to be shipped from manufacturer to end-user. Like most products, they are designed to withstand standard

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[13] Stability of SARS-CoV-2 on Critical Personal Protective Equipment, Samantha B Kasloff, James E Strong, Duane Funk, Todd A Cutts, medRxiv 2020.06.11.20128884; doi:https://doi.org/10.1101/2020.06.11.20128884

[14] The effect of temperature and humidity on the stability of SARS-CoV-2 and other enveloped viruses, Dylan H. Morris, Kwe Claude H. Yinda, Amandine Gamble, Fernando W. Rossine, Qishen Huang, Trenton Bushmaker, Robert J Fischer, M. Jeremiah Matson, Neeltje van Doremalen, Peter J Vikesland, Linsey C. Marr, Vincent Munster, James O Lloyd-Smith, bioRxiv 2020.10.16.341883; doi: https://doi.org/10.1101/2020.10.16.341883 This pre-print indicates less than a 45 minute half-life at 40C for a broad range of pathogens in a saliva matrix deposited on plastic test coupons.

shipping and storage conditions. Experimental studies[15] of containerized shipping containers and trucks indicate 50°C is a typical upper bound (with rare excursions to 60°C - note at higher temperatures, such as 70°C, N95 masks degrade[16]). Consistent with these findings, many product companies (including 3M for N95 masks[17]) recommend 50°C as the maximum safe storage and shipping temperature. While conservative (some PPE are more accepting of higher temperatures, and future decontamination-grade PPE may be more heat tolerant), we chose 50°C or below as likely to preserve PPE efficacy across all commercial products, irrespective of designs.

Humidity: To avoid the cost and complexity of humidity-controlled ovens, the WASP-D protocol draws in ambient air to gently ventilate the heated storage/decontamination chamber. Since the chamber is hotter than ambient, and hot air can "hold" higher levels of moisture and the relative humidity declines. Ventilation also dries out sweat, limiting mold and fungal growth. But until dried out, evaporative cooling lowers[18] PPE surface temperature, so the protocol compensates by extending the decontamination cycle.

Climate varies widely across the globe, so we considered three limiting cases- Boston, Rio de Janeiro and Lagos. By holding the partial pressure of ambient humidity constant as it warms to ventilate the chamber, we find:

Location	Median temperature	Relative humidity of ambient inlet air at median temperature	Relative humidity at 43°C during cycle
Boston	70°F (21°C)	60%	17%
Rio de Janeiro	81°F (27°C)	80%	22%
Lagos	90°F (32°C)	80%	44%

For our initial experiments, we chose 22%RH (@43°C) as a representative average.

There is some evidence[19] ambient humidity levels under certain conditions are protective to the virus, especially when the virus is contained in a saliva droplet. Again, WASP-D incorporates additional time as a "guard-band" in compensation.

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[15] https://www.tis-gdv.de/tis_e/containe/klima/klima-htm/; David Leinberger, Temperature & humidity in ocean containers. Technical report, Xerox Corporation, 2006.

[16] Fischer RJ, Morris DH, van Doremalen N, et al. Effectiveness of N95 Respirator Decontamination and Reuse against SARS-CoV-2 Virus. *Emerging Infectious Diseases*. 2020;26(9):2253-2255. doi:10.3201/eid2609.201524

[17] https://multimedia.3m.com/mws/media/1538979O/3m-disposable-respirator-1860-1860s-technical-data-sheet.pdf

[18] https://genuineideas.com/ArticlesIndex/stallbbq.html

[19] doi: 10.1021/acsnano.0c06565, **DOI:** 10.1128/AEM.02291-09, **DOI:** 10.1128/mSphere.00441-20

Pathogen Reduction: For safety and expediency, bovine coronavirus, a beta coronavirus like SARS-CoV-2, and MS-2 were chosen as surrogate viruses. As the EPA notes, "According to this hierarchy, if an antimicrobial product can kill a small, non-enveloped virus it should be able to kill any large, non-enveloped virus or any enveloped virus. Similarly, a product that can kill a large, non-enveloped virus should be able to kill any enveloped virus."[20]

Experimental Method:

Material testing. N-95 test coupons measuring approximately $1cm^2$ were placed into a single well of a 12-well dish, in triplicate. Over the course of two experiments, an average of 1.2×10^6 $TCID_{50}$ units of BCoV-mebus in minimum essential medium (MEM) containing artificial saliva[21,22] was spotted onto each test coupon. Each 12-well dish were placed in their respective temperature incubators for the desired time points. Following incubation, N-95 test coupons were incubated with one mL of medium and rocked at room temperature for ten minutes to recover infectious virus.

Bovine Coronavirus infectivity assay. Madin Darby Bovine Kidney (MDBK) cells were maintained in advanced minimal essential medium (AMEM, Gibco) supplemented with 5% heat-inactivated Fetal Bovine Serum (FBS), 2 mM L-Glutamine (Gibco), and 1% Antibiotic/Actinomycotic cocktail (Gibco) [23]. The BCoV-Mebus (GenBank: U00735.2) strain was used[24]. Median tissue culture infectious dose ($TCID_{50}$) assays were performed according to published protocols. To detect cytopathogenicity (CPE) caused by the virus, BCoV-infected and uninfected (control) MDBK cells were imaged in a SpectraMax Imaging Cytometer (Molecular Devices) at a 5-millisecond exposure. Negative controls, which included uninfected MDBK cells and MDBK cells incubated with MEM exposed to a N-95 test coupon, were negative for CPE induction. $TCID_{50}$ values were calculated using the Reed-Muench method[25].

MS-2 plaque assay. Plaque assays were performed using MS-2, a non-enveloped bacteriophage, to test its ability to survive and infect *E. coli* at the selected temperatures and

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[20] https://www.epa.gov/sites/production/files/2016-09/documents/emerging_viral_pathogen_program_guidance_final_8_19_16_001_0.pdf

[21] ASTM International. *E2197-17e1 Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals*. West Conshohocken, PA; ASTM International, 2017. doi: https://doi.org/10.1520/E2197-17E01

[22] ASTM International. *E1052-20 Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension*. West Conshohocken, PA; ASTM International, 2020. doi: https://doi.org/10.1520/E1052-20

[23] **https://doi.org/10.1002/9780471729259.mc15e02s37;** Hasoksuz M., Vlasova A., Saif L.J. (2008) Detection of Group 2a Coronaviruses with Emphasis on Bovine and Wild Ruminant Strains. In: Cavanagh D. (eds) SARS- and Other Coronaviruses. Methods in Molecular Biology (Methods and Protocols), vol 454. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-59745-181-9_5

[24] Benfield DA and Saif LJ, J Clin Microbiol. 1990 Jun;28(6):1454-7. doi: 10.1128/JCM.28.6.1454-1457.1990. *Cell culture propagation of a coronavirus isolated from cows with winter dysentery.*

[25] Lindenbach BD "Measuring HCV infectivity produced in cell culture and in vivo", Methods Mol Biol. (2009) 510:329-36

[24] Reference: Yang, L. et al. (2016). An improved plating assay for determination of phage titer. African Journal of Biotechnology. 15(23): 1078-1082.

time durations (*Escherichia coli* bacteriophage MS2 ATCC® 15597B1™). A saliva and MS-2 mixture was spotted onto each test coupon. MS-2 was recovered and eight, 10-fold dilutions were made. Ten microliters of each dilution were spotted, and an agar overlay was added[24]. After a 24-hour incubation period, plaques were counted, and the titer of MS-2 was calculated.

Results: After 12 hours at 43°C both MS-2 and BoCoV viral load are projected to decline by a factor of 10^6 (Figure 1). At room temperature (22°C) levels of infectious MS-2 and Bovine Coronavirus decline appears to slow. These results are in rough agreement with a variety of lower temperature measurements made by other groups under different conditions[14]. A viral load reduction of 10^6 is equivalent to 20 half-lives, so if the half-life at 43°F is around a half-hour, ten hours (20*0.5) will be sufficient to reduce by 10^6. While not definitive, given the approximately exponential decrease in life-time with temperature by about a factor of 3 per decade Celsius[25] , the WASP-D protocol recommends 46°C and 12 hours.

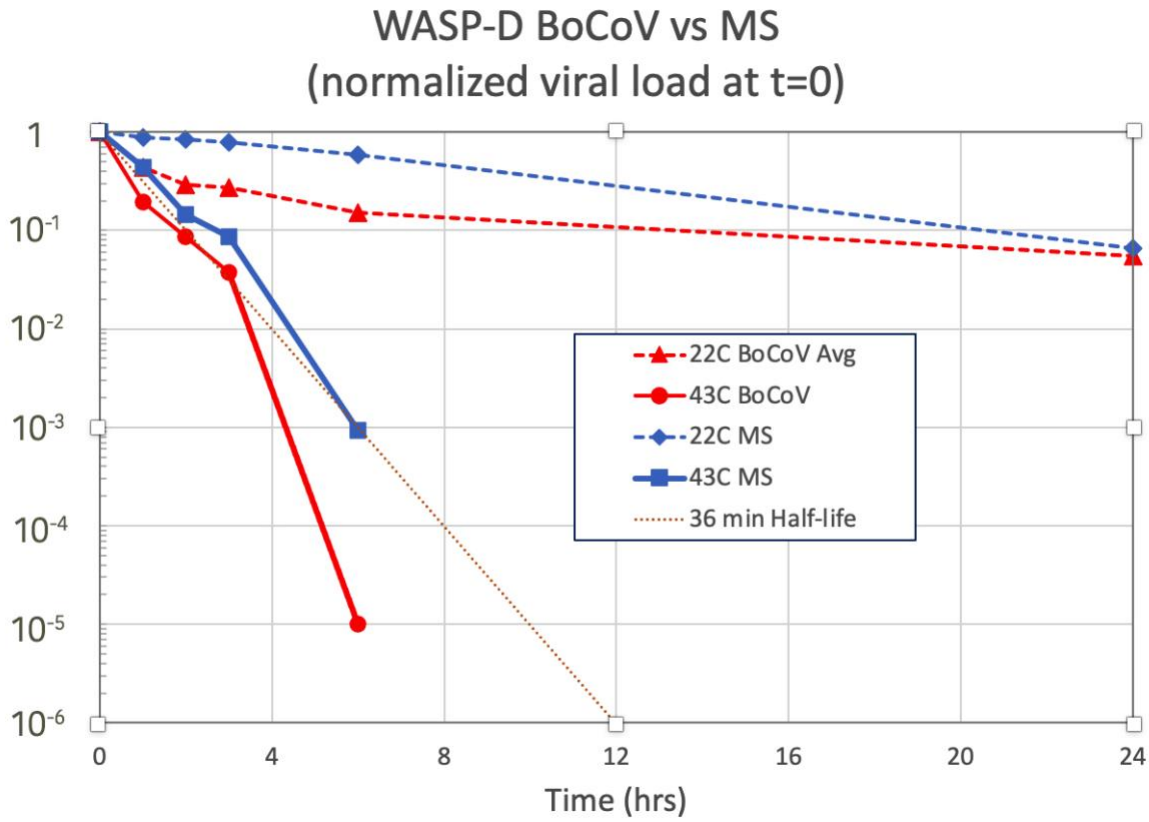

Figure 1 The 22°C Bovine Coronavirus (BoCoV) curve is an average of two experiments. MS = MS-2 bacteriophage. The dotted line denotes an exponential 12 hour reduction by 10^6.

		% Reduction vs control	
Exposure (in hours)	Temperature (°C)	Trial 1	Trial 2
0	22	N/A	N/A
0.25	22	24.910%	30.643%
0.50	22	41.440%	37.024%
1	22	49.898%	55.772%
2	22	65.657%	73.849%
3	22	70.249%	74.150%
6	22	92.117%	81.310%
24	22	93.919%	94.893%
0	43	N/A	N/A
0.25	43	37.024%	40.160%
0.50	43	48.479%	47.724%
1	43	86.876%	80.769%
2	43	90.401%	91.451%
3	43	96.261%	96.215%
6	43	99.999%	99.999%
24	43	99.999%	99.999%

Figure 2: Heat reduces Bovine Coronavirus infectivity. Bovine Coronavirus (strain Mebus) was heated at 22°C or 43°C for up to twenty-four hours. Following heat treatment, Bovine Coronvirus was incubated with MDBK cells for 48-hours, cytopathogenecity (CPE) was scored, and median tissue culture infectivity ($TCID_{50}$) was calculated. The $TCID_{50}$ assay has a limit of detection of 10 $TCID_{50}$ units (here defined as CPE detected in all four replicates. If we define limit of detection (LoD) as any of the replicates showing CPE, then the LoD is 1 $TCID_{50}$ units. No infectious virus was detected following exposure to 43°C heat for six or 24 hours.

Conclusions and recommendations: On BoCoV test samples and MS-2 e-coli vectors, WASP-D is expected to result in a 10^6 viral load reduction after 12 hours at 115°F=46°C and 22% relative humidity. We expect a similar effect on the human beta coronavirus SARS-CoV-2. While we have not yet field tested this methodology (studies on-going), WASP-D offers the prospect of saving lives around the globe by allowing for the relatively safe re-use of scarce PPE, and we hope others will confirm and extend our results and protocol at https://www.protocols.io/view/wasp-d-field-protocol-bkcskswe.

While the intended audience for WASP-D are LMIN, it does not escape our notice that domestic hospitals and individuals would benefit from a convenient method to decontaminate PPE, or even entire rooms and buildings. We can imagine colleges might employ this system to decontaminate student masks- or libraries, to sanitize circulating books and DVDs. We also emphasize, in this world of internet misinformation, **and in no uncertain terms**, that WASP-D cannot be used to decontaminate people. A steam spa, or blowing hot air up the nose, is ineffective and possibly dangerous. More subtly, exponential viral decay is exquisitely sensitive to time and temperature. **100°F and 8 hours is not a substitute for 115°F and 12 hours**. A home cooking oven set on WARM will overshoot the 125°F upper safe temperature range (due to radiation from the exposed heating elements) and possibly damage the PPE. Extra time must be allotted to account for the cooling effect of evaporating moisture, or low PPE thermal conductivity[26] .

[26] For example, if WASP-D is used to decontaminate books, a single book might take three hours to reach 43°C in a 46°C room, while a stack of books (due to the insulation value of cellulosic paper) might take ten hours or more. These heating time delays must be added to the 12-hour WASP-D cycle.

Prudent care must be taken when applying WASP-D to real-world situations. For more details including up-to-date improvements and caveats, please see https://www.protocols.io/view/wasp-d-field-protocol-bkcskswe .

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