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Early View

Original article

Viable virus aerosol propagation by positive airway pressure (PAP) circuit leak and mitigation with a ventilated patient hood

Shane A. Landry, Jeremy J. Barr, Martin I. MacDonald, Dinesh Subedi, Darren Mansfield, Garun S. Hamilton, Bradley A. Edwards, Simon A. Joosten

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Title: Viable virus aerosol propagation by positive airway pressure (PAP) circuit leak and mitigation with a ventilated patient hood

Subtitle: Virus aerosol propagation by PAP circuit leak

Authors:
Shane A Landry[1] PhD, Jeremy J Barr[2] PhD, Martin I MacDonald[3] PhD, Dinesh Subedi[2] PhD, Darren Mansfield[3,4,5] PhD, Garun S Hamilton[3,4,5] PhD, Bradley A. Edwards[1,2] PhD, Simon A Joosten[3,4,5] PhD

Affiliations:
1. *Department of Physiology, School of Biomedical Sciences & Biomedical Discovery Institute, Monash University, Melbourne, VIC, Australia*
2. *School of Biological Sciences, Monash University, Clayton, VIC, Australia*
3. *Monash Lung and Sleep, Monash Medical Centre, Clayton, VIC, Australia*
4. *School of Clinical Sciences, Monash University, Melbourne, VIC, Australia*
5. *Monash Partners – Epworth, Victoria, VIC, Australia*
6. *Turner Institute for Brain and Mental Health, Monash University, Melbourne, VIC, Australia*

Correspondence:
Author: Shane Landry, PhD
Tel: +613 9905 9767
Email: shane.landry@monash.edu
Address: Sleep and Circadian Medicine Laboratory
Ground Floor, Monash University BASE facility
264 Ferntree Gully Road
Notting Hill, 3168, Victoria, Australia

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TAKE HOME MESSAGE

This live virus model demonstrates that PAP mask leak maybe a major source of environmental contamination and nosocomial spread of infectious respiratory diseases. A simply constructed ventilated hood with HEPA filter is an efficacious countermeasure.

Characters: 256

ABSTRACT

Introduction Nosocomial transmission of SARS-CoV-2 has been a major feature of the COVID-19 pandemic. Evidence suggests patients can auto-emit aerosols containing viable viruses, these aerosols could be further propagated when patients undergo certain treatments including continuous positive airway pressure (PAP) therapy. Our aim was to assess i) the degree of viable virus propagated from PAP circuit mask leak, ii) the efficacy of a ventilated plastic canopy to mitigate virus propagation.

Methods: Bacteriophage PhiX174 (10^8copies/mL) was nebulised into a custom PAP circuit. Mask leak was systematically varied at the mask interface. Plates containing *Escherichia coli* host quantified viable virus (via plaque forming unit) settling on surfaces around the room. The efficacy of a low-cost ventilated headboard created from a tarpaulin hood and a high efficiency particulate air (HEPA) filter was tested.

Results: Mask leak was associated with virus contamination in a dose-dependent manner (χ^2= 58.24, df=4, p<0.001). Moderate mask leak (≥21 L/min) was associated with virus counts equivalent to using PAP with a vented mask. The highest frequency of viruses was detected on surfaces 1m away, however, viable viruses were recorded up to 3.86m from the source. A plastic hood with HEPA filtration significantly reduced viable viruses on all plates. HEPA exchange rates ≥170m^3/hr eradicated all evidence of virus contamination.

Conclusion: Mask leak from PAP may be a major source of environmental contamination and nosocomial spread of infectious respiratory diseases. Subclinical mask leak levels should be treated as an infectious risk. Low-cost patient hoods with HEPA filtration are an effective countermeasure.

Abstract Words: 250

Key words: Covid-19, aerosol, PAP, bacteriophage

INTRODUCTION

The COVID-19 pandemic has placed enormous pressure on public health and hospital systems across the globe. In the context of the ongoing health disaster, healthcare worker (HCW) furlough, morbidity and mortality has further stretched hospital resources. Those workers in roles caring for COVID-19 patients are at highest risk [1].

Positive Airway Pressure (PAP), applied either as continuous positive airways pressure (CPAP) or Non-invasive Ventilation (NIV) is life-saving treatment for patients with COVID-19 [2, 3]. Given that PAP usage can propagate patient expired air via exhalation ports [4], respiratory circuits are often modified to use a mask without vents ("non-vented mask") and to pass expired air through a viricidal filter prior to release from the circuit [5]. However, HCWs attending patients with severe COVID-19 who require PAP remain at increased risk of infection [6], even when personal protective equipment is utilised [7]. It is unclear why HCWs caring for patients on PAP are at higher risk. Inadequate protective equipment use [8] and increased virus exposure from NIV mask leak are clear possibilities – although no data exists on the extent to which mask leak presents an environmental contamination risk. Importantly, some degree of unintended mask leak is present in all situations where PAP is applied to a mask, and leak is much more likely when a high degree of pressure/ventilatory support is required.

There is mounting evidence that aerosols containing SARS-CoV-2 play an important role in nosocomial spread of COVID-19 infection. Patients with seasonal coronavirus infection generate and emit virus containing aerosol [9] and SARS-CoV-2 aerosols can remain infectious for at least three, and up to 72 hours after generation [10]. Even when patients are cared for in biocontainment units with negative-pressure rooms, SARS-CoV-2 can be detected in the air inside and outside the patient room, on surfaces distant from patients [11, 12] including non-clinical areas [13]. Due to the highly contagious nature of SARS-CoV-2 [14] and the high morbidity and mortality associated with COVID-19 [15-17], any environmental contamination poses a risk to HCWs and other patients. Given the limited availability of airborne infection isolation rooms (AIIR)/"negative pressure rooms", the United States Centres for Disease Control and Prevention (CDC) recommends the use of ventilated hoods with high efficiency particulate air (HEPA) filtration [18], although the effectiveness of these interventions at reducing HCW and environmental contamination has not been established.

We aimed to quantify the amount of viable virus that is propagated from clinically relevant levels of PAP circuit leak. To accomplish this, we used the surrogate virus PhiX174 (*Microviridae* family), which is a tail-less, icosahedral, non-enveloped, bacteriophage with a linear ssDNA genome. Due to its small size (0.025μm) and intrinsic stability, PhiX174 is commonly used as a viral aerosol model [19, 20]. Finally, we determined if a simple patient hood with a commercial HEPA filter set to different air flow exchange rates can mitigate environmental spread of viable virus aerosol.

METHODS

A series of experiments were designed to quantify aerosolised viral propagation of a simulated patient with viral respiratory disease (e.g. COVID-19) undergoing PAP with a non-vented mask in a non-negative pressure hospital room. *It was assumed that the airflows contained by a PAP mask contain aerosols containing viable virus.*

Bacteriophage PhiX174

PhiX174 was propagated using the bacterial host *E. coli C* ATCC13706 grown in Tryptic Soy Broth (TSB). The lysate was purified following the Phage-on-Tap protocol [21] and resuspended in 1X phosphate-buffered saline (PBS; Omnipur, Gibbstown, NJ, USA). Phage titre was determined by soft agar overlay method. To quantify viral spread, a series of soft agar plates containing *E. coli C* bacterial host (Figure 1A) were positioned and left uncovered in a sealed room for set periods of time (locations shown in Figure 1B). Settle plates were then sealed, incubated overnight at 37 °C and viral plaques were enumerated the following day.

A series of detection sensitivity experiments were performed to find the optimal phage dose required for optimal detection on settling plates. These experiments were informed by known quantities of seasonal coronavirus viral copies emitted by the upper airway of ambulant/non-hospitalised patients [9]. A nebulised 10mL solution of 10^8 PhiX174 virions per mL (for an effective total dose of 10^9 phages) provided ideal detection sensitivity. Details of the titration experiments can be found in the supplement.

Aerosol generation

Aerosols were generated using a nebuliser (Pari-PEP, PARI Respiratory Equipment, VA, USA). Medical air (9 L/min) was delivered to the nebuliser via tubing connected to a wall mounted flow metre (RTM3 0-15 L/m). The Pari-Pep device produces a distribution of aerosol particle size of 3.42 ± 0.15 µm [22].

Simulated circuit leak

A respiratory circuit was created to generate stable and discrete levels of PAP circuit leak (Figure 2). The circuit comprised a sealed end piece connected to 3 pressure port connectors (Fisher & Paykel, 900HC452) connected in series. These closable pressure ports (6 total) served as ‘leak ports’ in the

circuit. Oxygen tubing was connected to each leak port and threaded through the elbow of a PAP mask (Resmed, Quattro) and taped to the edges of the mask. Each tube/port was fixed in place to direct leak towards typical areas of mask leak. Connected in order from the mask to machine, two T-piece connectors were placed in series, the first was connected to the Pari-Pep Nebuliser which served as the aerosol input point, the second was (Fisher & Paykel, RT017) was attached to a viral filter (SureGard, RJVKB6, Viral Filtration Efficiency=99.99%, tested against PhiX174) which served as the filtered expiratory vent. The circuit was attached via CPAP hosing (Fisher & Paykel, 900HC221) to a pressure source (Phillips-Respironics, P_{crit} 3000). Given that our primary aim was to determine how leak influences aerosolised virus dispersion, we chose to deliver PAP in the form of CPAP rather than bi-level (Bi-PAP) as it can be engineered to provide a continuous and more easily controlled leak profile.

CPAP pressure 15.5 cmH_2O paired with 9 L/min nebuliser air input produced approximately 7 L/min leak increments for each port open such that 0, 7, 21, 28, 42 L/min leak could be generated by opening 0, 1, 3, 4 and 6 leak ports respectively. These leak levels were tested and calibrated quantitatively and were repeatable (Supplement Table S2). These levels were chosen because they represent leak likely to be experienced clinically.

Clinical Room

All experiments were undertaken in a room with dimensions: 4.0 × 3.25 × 2.7 m (surface area 13.0 m^2, volume 35.1 m^3), Figure 1B. All entrances and vents were taped shut and heating and cooling appliances were switched off. The room was insulated, with continuously recorded temperature (M±SD = 21.4±1.0 ºC) and barometric pressure (758.3±2.5 mmHg) varying minimally during experimental procedures. The room was furnished with a single hospital bed, table, and chair.

Ten settling plates were placed at specific sites around the room to quantify viruses settling on surfaces. Three plates were mounted to hang at head height perpendicular to the floor facing the source. Two settling plates were within 1.0 metre of the bedhead/aerosol source, four plates (3 settling, 1 hanging) were within 1.5 metres, with the remaining plates being between 1.5 metres and 3.86m (exact distances provided in supplementary Table S1).

CPAP hosing and oxygen/air tubing were fed into the room from an external control room (via sealed holes in the wall). The nebuliser and leak circuit were taped to the head of the bed and placed so that the leak outputs were positioned where a patient's head would normally be.

HEPA filtration and patient hood structure

An air-purifier with HEPA filter (IQ Air, Healthpro 250) was used to perform multiple air exchanges to clear the room of virus between experiments. Pilot testing (see supplementary materials) showed that the room could be adequately cleared by 30 minutes of run time at a flow rate of 470m^3/hr (approximately 6.7 exchanges).

To test protective measures designed to reduce viral propagation, a hood structure was created modelled on CDC recommendations [18], using hardware store materials (cost ~$40AUD/$29USD). The structure draped over the top of the bed enclosing the patient's head and the air intake of the air-purifier (Figure 3).

Experimental Protocols:

Experiment 1: To assess the degree of viable viral aerosol propagation associated with PAP circuit leak, the bacteriophage lysate was nebulised for 45 minutes into the leak circuit, which was pressurised at 15.5 cmH_2O. Plates were covered and removed at the end of the 45 minute period. This was repeated three times for each leak level (0, 7, 21, 28, 42 L/min). As a comparative control condition, the viral filter was removed from the expiratory limb of the circuit (equivalent to using a vented PAP mask) and the mask leak was set to 0 L/min. Between each condition the air purifier was run (at 470m^3/hr) for 30 minutes. After, control plates were placed in the room for 10 minutes to ensure room air was free of virus.

Experiment 2: To assess the ability for a protective hood and HEPA filter to reduce aerosolised virus propagation and environmental contamination, the bacteriophage lysate was first nebulised for 30 minutes in the room (unconnected to the PAP circuit). Plates were covered and replaced at 30, 45, and 60 minutes post-nebulisation by one of the investigators who remained in the sealed room. These conditions were then repeated with the nebuliser placed within the hood, then repeated with the air purifier (within the hood) turned on at 50, 170 and 470m^3/hr exchange settings.

Data analysis:

In each experiment viable viruses were quantified by counting the number of plaques on settling plates. Plaque counts >200 were considered too-many-to-count (TMTC) and were rated using an ordinal visual rating scale (+, ++, +++, ++++), with TMTC++++ indicating that complete lysis has occurred on the plate. For graphing and analysis purposes TMTC ratings were given numeric values of 200, 210, 220, and 230. Friedman's test with post hoc comparisons (Dunn test) was used to compare plaque counts between conditions. A p value >0.05 was considered statistically significant.

RESULTS

Viral aerosol propagation associated with PAP circuit leak

Figure 4 shows the degree of aerosolised virus escaping from the PAP circuit with non-filtered, non-sealed circuit (at 0 L/min leak) as a reference. Increased leak was associated with increase in viral counts across settling plates in a dose-response manner (χ^2= 58.24, df=4, p<0.001). Post hoc tests showed viral counts were significantly lower in the 0 L/min condition compared to any other leak level. Similarly, viral counts were higher in the 42 L/min leak condition compared to any other level of leak. Mask leak levels ≥21 L/min demonstrated comparable viral counts to when the viral filter was removed from the expiratory vent of the circuit.

Plates 4 and 5, which were located less than 1m from the leak point, consistently showed the highest plaque counts and were frequency TMTC. The three hanging plates tended to have lowest plaque counts across all leak levels.

To assess the relationship with and distance, the viral counts (combined across all leak conditions) were plotted against the distance of each individual plate from the leak source. Shown in Figure 5 there was an inverse relationship ($r_{spearmans}$= -0.166, p=0.02, n=195) with viral counts decreasing with distance from source. Notably the most distant plate (3.86m) had consistent viral counts at all leak levels ≥7 L/min.

Efficacy of Hood and HEPA filter

When the bacteriophage solution was nebulised directly into the room without the leak apparatus, high viral counts were found on all settle plates, with significant aerosolised viral load persisting up to 60 minutes after the solution had been completely nebulised (Figure 6). Comparatively, hanging plates (positions 6, 7 and 11) counts remained relatively low. When viral aerosolization was repeated with the addition of the hood structure, viral counts were significantly attenuated. The further addition of the HEPA filter had minimal additional efficacy at 50m^3/hr, however at HEPA settings of 170m^3/hr and 470m^3/hr all plates registered zero viral counts at all time points.

DISCUSSION

Using a viable virus model of aerosolised nosocomial transmission, our study quantified the propagation risk associated with unintended PAP system leak, and the efficacy of a hood and HEPA filter containment structure to mitigate environmental contamination. Our data shows that aerosols containing viable virus, in similar concentrations to those generated by patients [9], can escape from PAP system leak and settle onto surfaces at least 3.68m away from the leak source – even at subclinical levels of leak (7 L/min). The degree of leaked virus-containing aerosols settling throughout the room is proportional to the amount of leak in a dose-dependent manner. Enclosing the head of the bed in a cheaply constructed hood, of plastic sheet and PVC piping, substantially attenuated the degree of virus spreading. Moreover, application of a HEPA filter (to the hood) at an exchange rate of $170m^3$/hr or greater, eliminated all evidence of virus spreading in the environment, even when 10^9 viruses were directly nebulised. We believe these findings have immediate and wide-reaching implications for the protection of HCWs on the front line of the COVID-19 pandemic.

Several studies have elucidated the risks currently facing HCWs in the COVID-19 pandemic. Leung et al. [9] demonstrated ambulatory non-hospitalised patients with seasonal coronavirus are capable of self-generating virus containing aerosol at rates of 10^5 copies/30mins, including by breathing without coughing. We also know that "superspreader" transmission dynamics, where a small proportion of cases are responsible for large numbers of transmissions, are a feature of previous SARS epidemics [23] and the COVID-19 pandemic [24]. This implies every COVID-19 patient is a potential silent virus aerosol generator. Santarpia et al. [11] demonstrated that even in a dedicated biocontainment facility with negative pressure rooms and hallways, SARS-CoV-2 can be detected on surfaces, including underneath the patient's bed, and at even higher concentrations in air samples from the room and hallway. The presence of such extensive contamination indicates that negative pressure do not completely eliminate the route of virus aerosol contamination. van Doremalen et al. [10] demonstrated SARS-CoV-2 aerosols can remain viable in the environment for between three and 72 hours. These studies demonstrate that infected patients generate aerosols which are an important part of extensive environmental contamination, even in dedicated specialised environments, and that environmental contamination by aerosols creates potential viable virus risks for HCWs.

Previous studies have demonstrated the potential for aerosol propagation from respiratory circuits. Using smoke and lasers to visualise localised aerosol particle spread, Hui et al. [4, 25] have shown typically vented CPAP/NIV masks produce (intended) pressure dependent leakage plumes from their exhalation ports in a 1 metre radius. While techniques that visualise particle spread quantify zones of high risk/density environmental contamination, expelled aerosols can travel substantially longer distances than 1 metre where they settle in the environment. Additionally, smoke and particle studies cannot assess the biological aspect of aerosol propagation risk. We have demonstrated that viable virus can be propagated by a respiratory circuit and remain viable in the environment – where it poses

a substantial risk for nosocomial transmission. Moreover, virus contained in aerosols were shown to impact on plates at head height, and settle on all surfaces, including the most distant point in the room (3.68 metres from the source). That virus is detectable at distances of greater than 3 metres from the source in our experiments raises important concerns for large open areas such as intensive care units and cohorted wards.

This is the first study to systematically examine virus aerosol propagation associated with unintended mask leak from a PAP system. Pressurised mask systems are prone to leak that can be difficult to detect at levels <10 L/min. High pressure requirements, mask interfaces with large contact surface-area, coughing, and facial wrinkles/skinfolds are all associated with increased mask leak. Importantly, mask leakage bypasses viricidal filters placed on the expiratory limb of a PAP circuit. This is a potential hazard that all non-invasive methods for delivering PAP are susceptible to (nasal, oronasal, full face masks, and helmet interfaces). Our study demonstrates that mask leak directly leads to viable virus aerosol propagation in a dose-dependent manner, suggesting that even clinically undetectable levels of leak could be a significant source of risk for HCWs, particularly with prolonged exposure. Of note, our experiments quantified the risk associated with short-term use (30-45mins) of PAP, however many patients may require substantially longer periods of use (>24 hours), which multiplies the associated risk.

The CDC predicted that during a pandemic demand for airborne infection isolation rooms would outstrip supply [26]. In this context, the CDC has guides for constructing ventilated headboards [18]. Several studies have demonstrated efficacy of ventilated hoods at capturing aerosols [27, 28]. However, our study is the first to demonstrate the ability of such a structure to eliminate viable virus propagation and environmental contamination. Our study builds on previous data to show even highly contaminated aerosols (containing 10^9 virus copies) can be eliminated by an apparatus modelled on CDC recommendations. Moreover, we show that this design can be equally effective when constructed from readily available low-cost materials, and with modest HEPA air exchanges rates ($\geq$170m^3/hr). Given that we are able to eliminate environmental contamination with modest exchange rates raises important considerations. Santarpia et al. [11] showed that even in the absence of AGPs, COVID-19 patients managed in a biocontainment facility with negative pressure at 12 exchanges/hour exhibited extensive environmental contamination including in the air and under the beds, An advantage of using our technique compared to a standard negative pressure room is the use of "point of emission" air exchange. A modest air filtration rate for an air purifier (170m^3/hr) within a relatively small hood over the patient's head could achieve rapid air exchange at the area of aerosol generation/propagation. We believe the hood structure contains/shepherds aerosol particles and fosters development of a wind tunnel that channels aerosol directly into the air purifier – a feature that is lacking in whole room negative pressure air exchanges.

Our methodology has several advantages over previous literature in this field. Bacteriophage PhiX174 has been used in several industrial and clinical applications (e.g. testing water and hospital filters). PhiX174 is harmless to humans and is of similar size (~0.025µm [29]) as SARS-CoV-2 (0.060-0.14µm). Using *E. coli* settling plates enabled us to detect the presence of viable virus with high resolution, in that a single viable copy of the virus causes a visible plaque to be formed on the *E. coli* lawn where the virus has lysed the bacterial host. In this way, our method is an extremely sensitive measure of viable virus propagation and settling in the environment. Furthermore, we have engineered a PAP circuit that can systematically assess how unintended system leak contributes the virus spreading. In this way the dose-response relationship between virus counts and virus settling is broadly generalisable to different mask interfaces and PAP types (CPAP, BiPAP, NIV).

This study, however, has several limitations. First, we used a nebuliser which produces a tight range of particle size (3.42µm ± 0.15µm) to produce virus containing aerosols. In contrast, aerosols generated by individuals when speaking or breathing are of similar magnitude [30], but present as a larger range of particle sizes including larger droplet ranges. Larger droplets settle faster and are less likely to travel long distances. Second, we aerosolised larger numbers of viruses than what has been currently shown to be emitted as aerosol by infected individuals when breathing (10^9 vs 10^5 [9]). However, these levels are well balanced by other factors. The settling plates sample approximately ~0.6% of the surface area, indicating that virus counts underestimate the total virus settling on surfaces by a factor of 10^2. Furthermore, our leak protocol assessed aerosol dispersion and settling over a relatively short window (45 min), whereas many patients may be expected to receive PAP for 10 times this length of time (underestimate factor of ~10^1). Therefore we believe that after adjusting for methodological factors which bias toward underestimating virus settling, we have used an acceptably plausible viral load to represent patient emissions in this study . Further discussion related to the number of viable viruses settling for each given leak is provided in the supplementary materials (Table S3). Importantly with regard to our hood/HEPA experiments, nebulizing 10^9 phages directly into the room most likely represents a 'worst-case' clinical scenario. Accordingly, our data showcases the extremely high efficacy of the hood and HEPA filtration structure to mitigate infection risk from patient emitted aerosolised virus, including the enhanced risk posed by a leaking PAP circuit.

In summary, our results demonstrate that unintended mask leak from PAP therapy can be a source of environmental contamination which can be mitigated a hood and HEPA filter. The hood and portable HEPA filter may represent a relatively low cost and portable adjunct to HCW protection from nosocomial COVID-19 transmission.

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FIGURE LEGENDS

Figure 1. Measuring viral dispersion. A. Photo of a setting plate with visible plaques. Each plaque indicates a single bacteriophage had settled in that precise location. **B.** Room layout with locations of 13 numbered plates shown in blue. Circular symbols represent where plates were positioned on floor or bed, rectangular symbols show plates hanging from ceiling oriented perpendicular to the floor.

Figure 2. PAP Leak circuit. A. Leak circuit Diagram. PhiX174 is nebulised directly into pressurised (15.5cmH_2O CPAP) circuit. Air escapes from the expiratory port (intended leak) which is covered by an anti-viral filter. Leak ports generate ~7 L/min leak for each port open. This simulated mask leak is fed via tubes into a PAP mask frame. **B.** Photograph of the leak circuit (with nebuliser attached), image shows six leak tubes connected to the mask indicating it is configured to produce leak at 42 L/min. **C.** PAP mask with leak tubes. Red arrows show the 6 locations where air can escape.

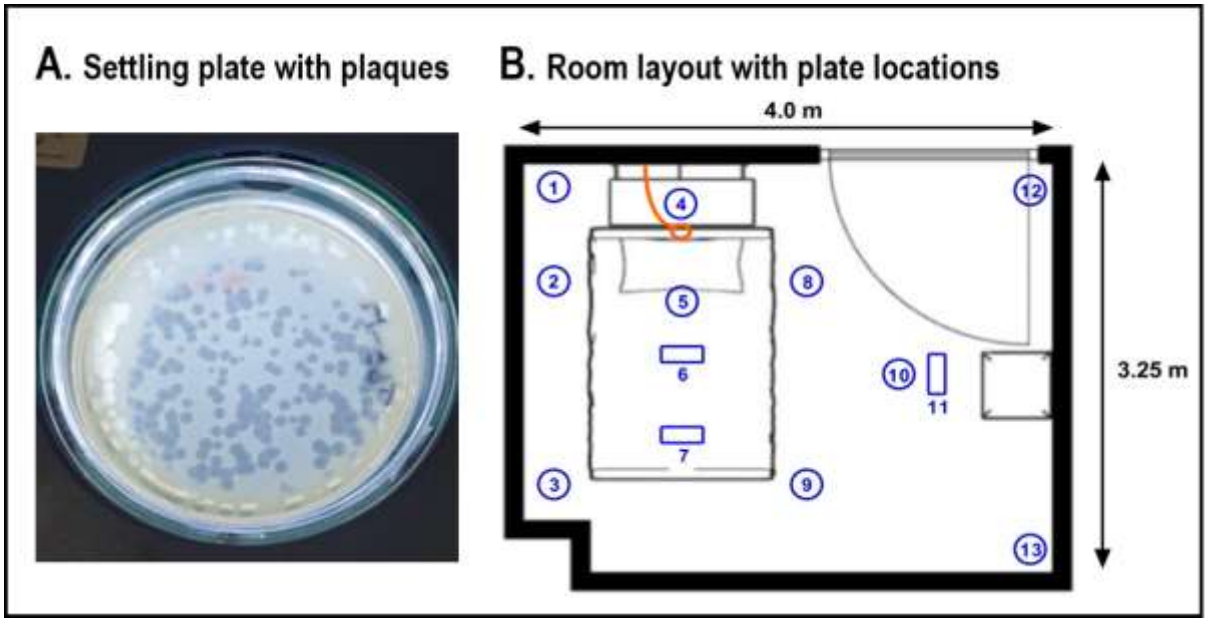
Figure 3. Patient Hood Structure. A. The hood structure was constructed out of a tarpaulin sheet with a square shaped hole (shaped to the size of the HEPA filter) cut in the middle. The hole was then placed over the HEPA filter as a skirt and taped in place. The tail end of the tarp was tucked under the bed mattress, and the front end of the tarp was draped over the top part of the bed. A square frame created from PVC piping was placed midway down the bed. The draping tarp was clipped into place over the frame with bulldog clips. Our intent was to use materials that were cheap and easily acquirable, as such the total cost of this structure was < $40 AUD. Photographs show structure from the front (**B**), side (**C**) and rear (**D**).

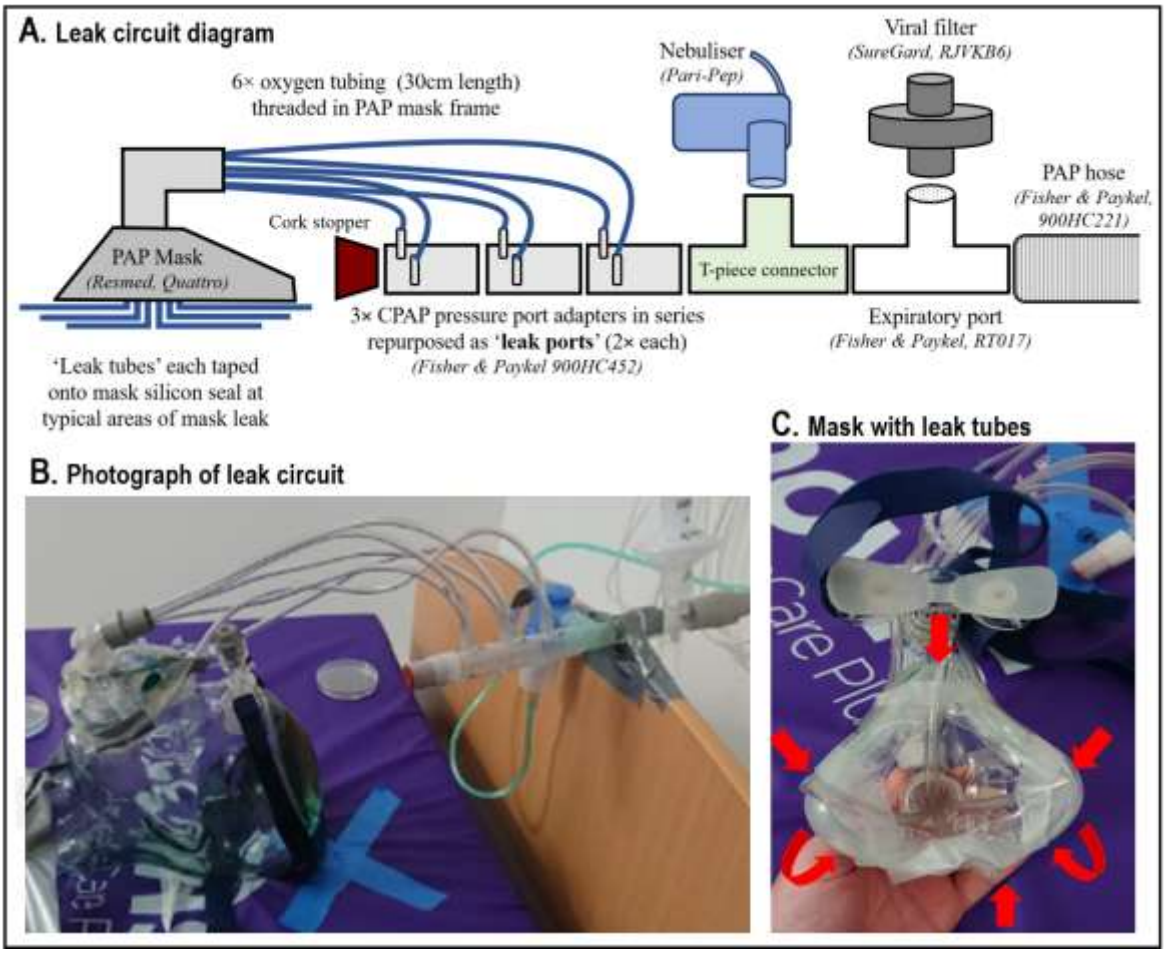
Figure 4. Viral aerosol propagation associated with PAP circuit leak. PhiX174 bacteriophages were nebulised over 45 minutes into in a pressurised (15.5cmH_2O) PAP circuit designed to leak at either 0, 7, 21, 28 or 42 L/min. As a comparator, the viral filter was removed from expiratory vent and the circuit set to 0 L/min leak in order to simulate a non-sealed mask (data highlighted in orange). These data show that virus settling in the environment increases with leak in a dose-dependent manner. Mask leak values greater than ≥21 L/min spread similar amounts of virus to the environment as an unsealed mask system (a known infection risk factor). Symbols represent viral counts from settling plates. Solid black circles represent plates 1m from the leak source, triangles represent hanging plates. Orange lines with error bars show median and interquartile range. Virus counts >200

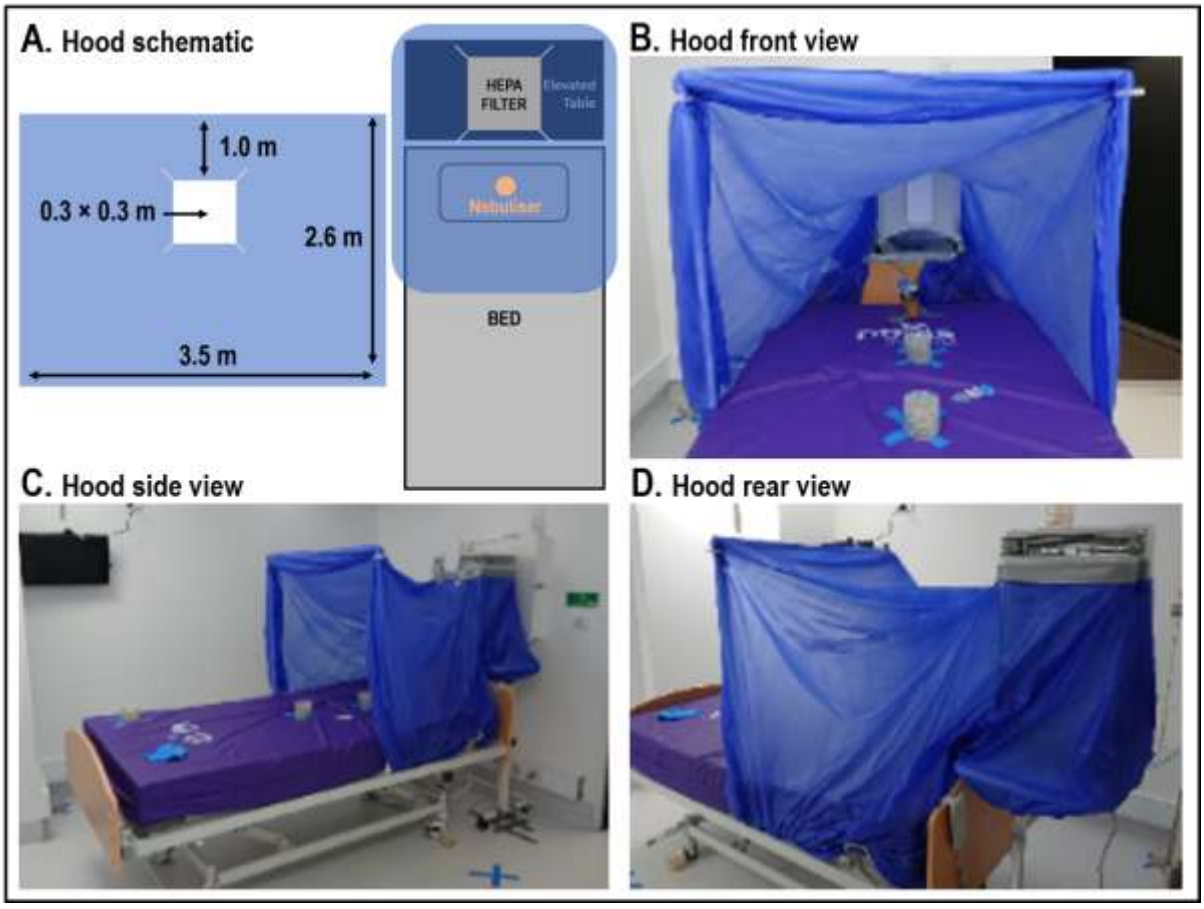
were considered too-many-to-count (TMTC) and were rated on using an ordinal (+, ++, +++, ++++) visual rating scale.

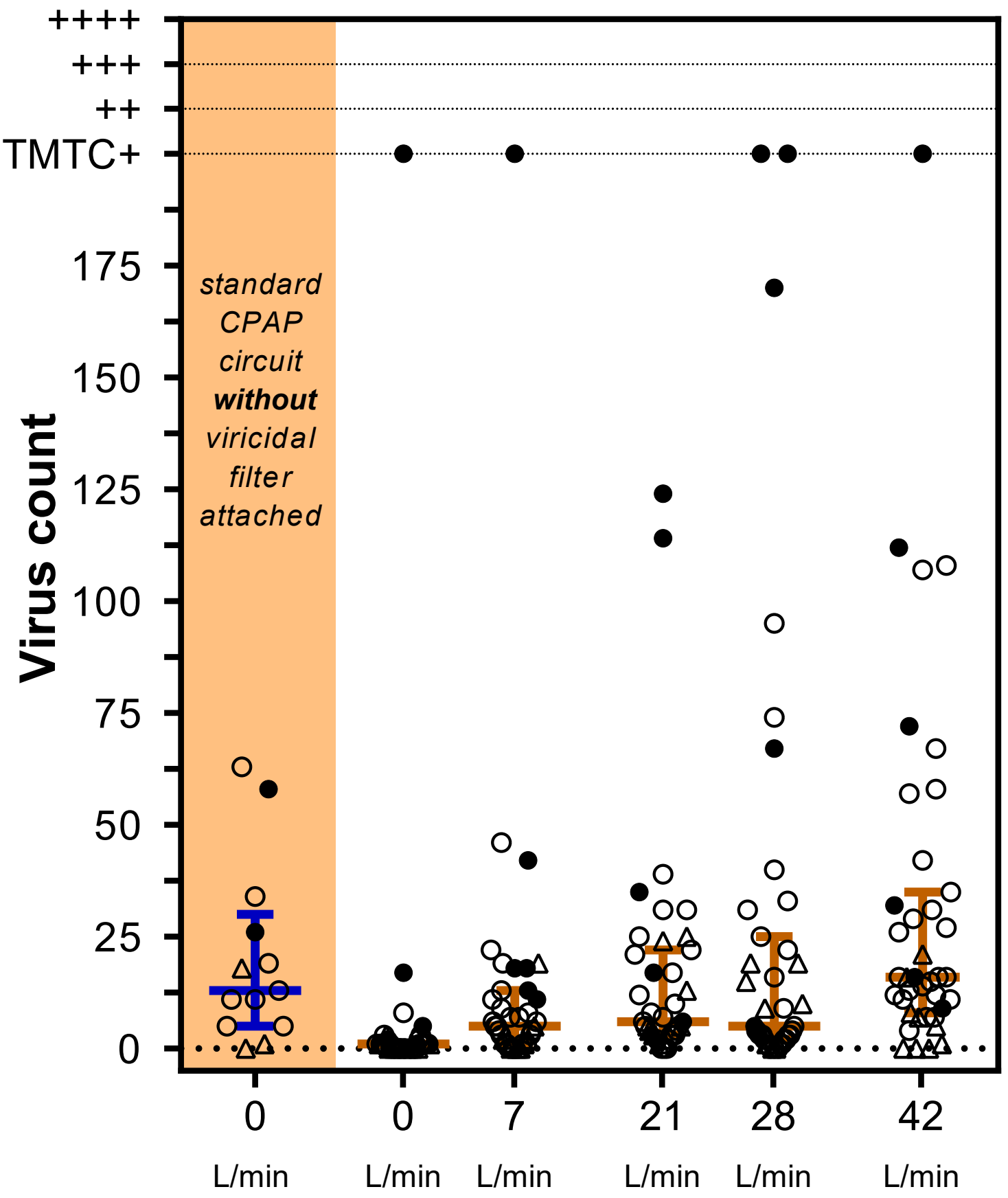
Figure 5. Viral counts versus distance from leak source. Viral counts for each plate, combined across all leak conditions are plotted according to the distance of the plate from the leak source. Specifically, the horizontal and vertical distances of the plate from the leak source were measured and the hypotenuse calculated according to Pythagoras' theorem. Solid black circles represent plates within 1m from the leak source, triangles represent hanging plates. Virus counts >200 were considered too-many-to-count (TMTC) and were rated on using an ordinal (+, ++, +++, ++++) visual rating scale

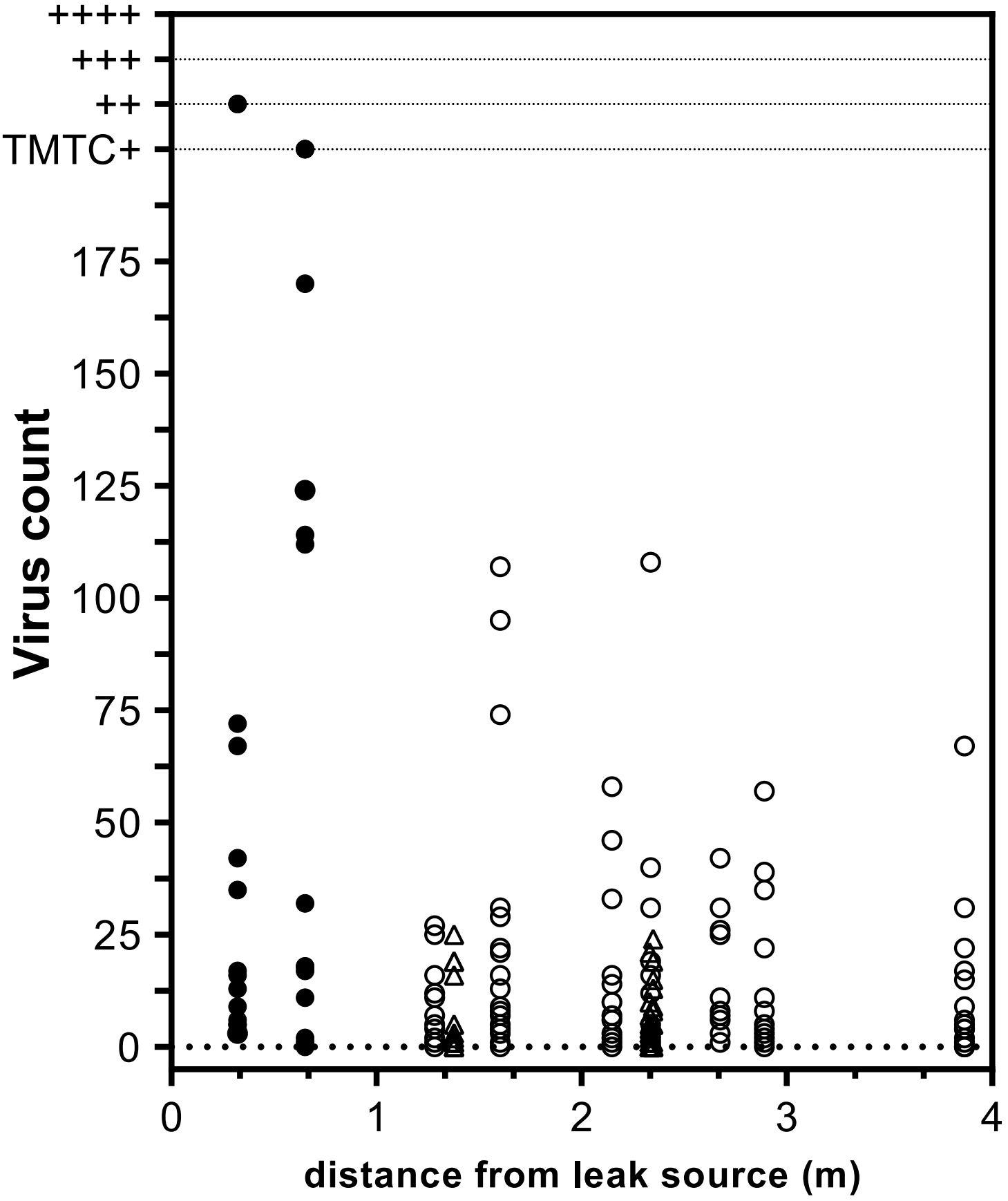
Figure 6. The efficacy of Hood and HEPA filter to reduce viral aerosol spread. PhiX174 bacteriophages were nebulised over 30 mins. Symbols represent plaque counts from settling plates shown across 3-time intervals (0-30, 30-45, 45-60 mins post-nebulisation). A hood structure fitted over the head of the bed substantially attenuated viral counts compared to no hood. No plaques were detected on any plates when HEPA filter was set to 170 or 470m^3/hr. Solid black circles represent plates within the hood (<1m from nebuliser), triangle represent hanging plates. Orange lines with error bars show median and interquartile range. Virus counts >200 were considered too-many-to-count (TMTC) and were rated using an ordinal visual rating scale.

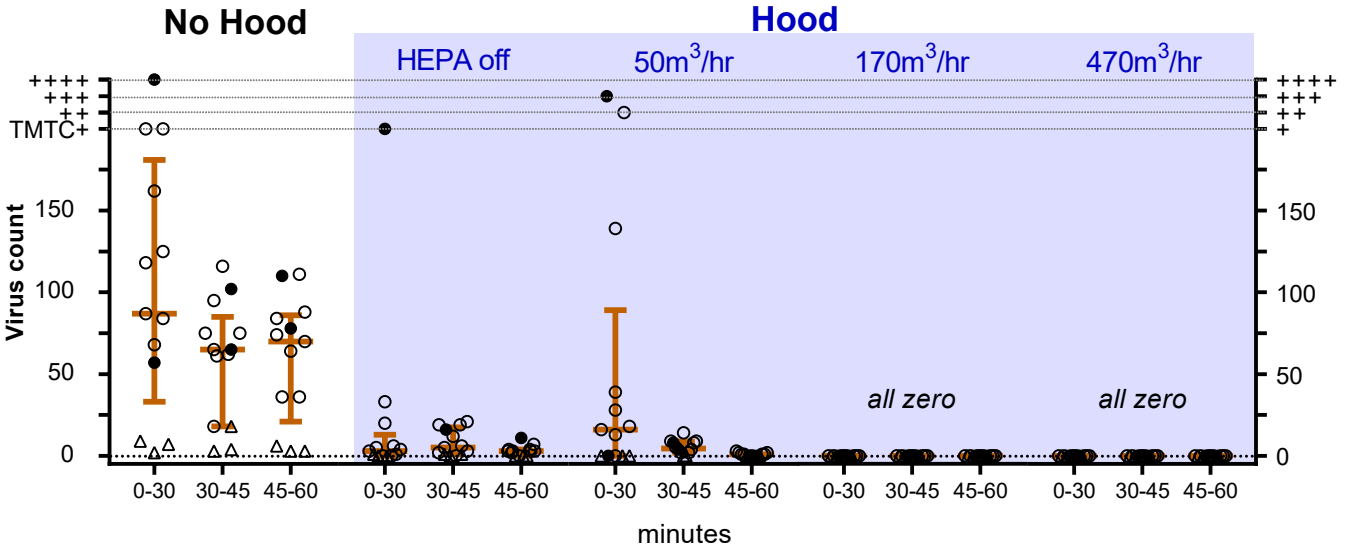

A. Settling plate with plaques
B. Room layout with plate locations
4.0 m
3.25 m
1
2
3
4
5
6
7
8
9
10
11
12
13


A. Leak circuit diagram
6× oxygen tubing (30cm length) threaded in PAP mask frame
Nebuliser (Pari-Pep)
Viral filter (SureGard, RJVKB6)
PAP hose (Fisher & Paykel, 900HC221)
Cork stopper
PAP Mask (Resmed, Quattro)
T-piece connector
3× CPAP pressure port adapters in series repurposed as 'leak ports' (2× each) (Fisher & Paykel 900HC452)
Expiratory port (Fisher & Paykel, RT017)
'Leak tubes' each taped onto mask silicon seal at typical areas of mask leak
B. Photograph of leak circuit
C. Mask with leak tubes


A. Hood schematic
1.0 m
0.3 × 0.3 m
2.6 m
3.5 m
HEPA FILTER
Elevated Table
Nebuliser
BED
B. Hood front view
C. Hood side view
D. Hood rear view


++++
+++
++
TMTC+
175
150
125
100
75
50
25
0
Virus count
standard CPAP circuit without viricidal filter attached
0
0
7
21
28
42
L/min
L/min
L/min
L/min
L/min
L/min


++++
+++
++
TMTC+
175
150
125
100
75
50
25
0
Virus count
0
1
2
3
4
distance from leak source (m)


No Hood
Hood
HEPA off
50m3/hr
170m3/hr
470m3/hr
++++
+++
++
TMTC+
+
Virus count
150
100
50
0
all zero
all zero
0-30
30-45
45-60
minutes

SUPPLEMENTRY MATERIALS

Viable virus aerosol propagation by PAP circuit leak and mitigation with a ventilated patient hood

Authors:
Shane A Landry[1] PhD,
Jeremy J Barr[2] PhD,
Martin I MacDonald[3] PhD,
Dinesh Subedi[2] PhD,
Darren Mansfield[3,4,5] PhD,
Garun S Hamilton[3,4,5] PhD,
Bradley A. Edwards[1,2] PhD,
Simon A Joosten[3,4,5] PhD

Affiliations

1. *Department of Physiology, School of Biomedical Sciences & Biomedical Discovery Institute, Monash University, Melbourne, VIC, Australia*
2. *School of Biological Sciences,* Monash University, Clayton, VIC, Australia
3. *Monash Lung and Sleep, Monash Medical Centre, Clayton, VIC, Australia*
4. *School of Clinical Sciences, Monash University, Melbourne, VIC, Australia*
5. *Monash Partners – Epworth, Victoria, VIC, Australia*
6. *Turner Institute for Brain and Mental Health, Monash University, Melbourne, VIC, Australia*

Correspondence:
Author: Shane Landry, PhD
Tel: +613 9905 9767
Email: shane.landry@monash.edu
Address: Sleep and Circadian Medicine Laboratory
Ground Floor, Monash University BASE facility
264 Ferntree Gully Road
Notting Hill, 3168, Victoria, Australia

SUPPLEMENTARY METHODS & RESULTS:

Room layout and plate distances

All experiments took place in a sealed laboratory suite at the Monash University BASE Facility. In order to assess virus dispersion, 13 sampling locations were chosen and marked with masking tape (see Figure S1). At each location a soft agar plate containing *E. coli C* bacterial host were left uncovered a during each experimental condition/time-point to assess the amount of viable virus setting on that surface.

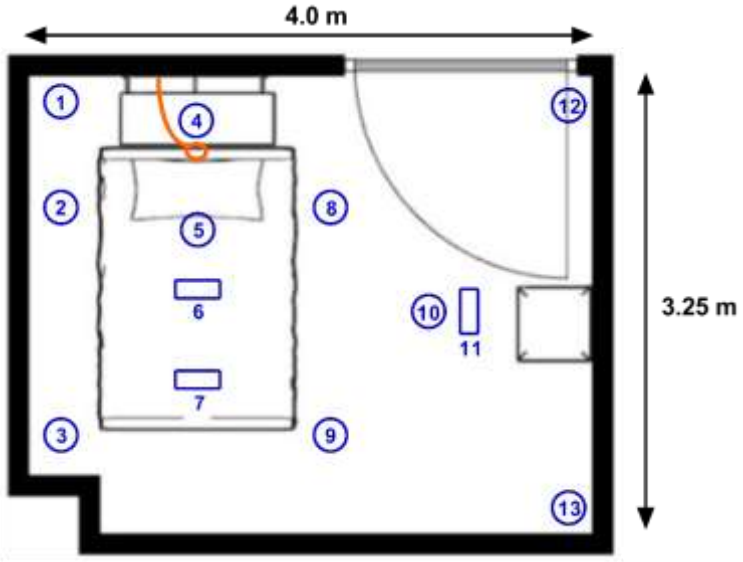


Figure S1: Room layout and plate locations.

Plates 1, 2, 3, 8, 9, 12 and 13 were positioned on the Floor. Plate 4 was mounted on a table behind the bedhead at approximately bed height. Plate 5 was mounted on the bed just below were a pillow would rest. Plates 6, 7 and 11 were hanging from the ceiling, oriented perpendicular to the ground at head height. The distance (x, y, z) of each of the plate locations from the bed head is shown in table S1. Aerosolised bacteriophages were emitted from nebuliser which was taped to the bed head (Figure 1, shown in orange). In experiments assessing PAP leak, the leak circuit was mounted on the bedhead with the mask interface/leak point positioned where the bed pillow would be placed (no pillow was actually present).

Table S1: Distances of plates from nebuliser/leak source

Plate	**X distance (cm)** *relative to centre of bed head*	**Y distance (cm)** *relative to bed head*	*relative to floor*	**Z distance (cm)** $Z = \sqrt{(x^2+y^2)}$
1	127	-98	0	160.42
2	127	-98	0	160.42
3	249	-98	0	267.59
4	30	-12	86	32.31
5	61	-23	75	65.19
6	98	97	195	137.89
7	214	97	195	234.96
8	83	-98	0	128.43
9	191	-98	0	214.67
10	212	-98	0	233.56
11	212	97	195	233.14
12	272	-98	0	289.12
13	374	-98	0	386.63

Leak circuit calibration

In order to test the leak properties of the circuit, a pneumotachograph (Hans Rudolph, model 3700A) was connected in series to the leak circuit (proximal to the leak ports and Pari-pep nebuliser, but distal to the expiratory port), see Figure S4. CPAP flow and the constant flow of medical air (9 L/min) through the nebuliser, was delivered to the circuit. Sequentially opening each of the leak ports generated a cumulative flow increase across the pneumotachograph proportional to the amount of air escaping (leaking) through each port.

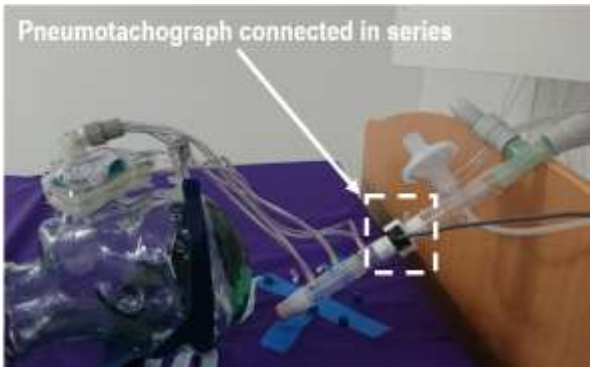


Figure S4. Leak settings validated using Pneumotachograph.

A CPAP level of 15.5 cmH_2O paired with 9 L/min nebuliser air input produced approximately 7 L/min of leak for each port opening. While pressurised, leak ports in the circuit were sequentially opened and the leak level associated with each was assessed on two separate occasions. The pneumotach and pressure signals were connected to a Power1401-3A amplifier and visualised in Spike2 software (Cambridge Electronic Design, CED). Data are shown in Table S2.

Table S2. Leak circuit test.

	Leak (L/min)		
Number of ports open	**1st Test**	**2nd Test**	**Mean ± SD**
0	0.0	0.0	0.0 ± 0.0
1	7.0	7.1	7.0 ± 0.04
2	14.3	14.5	14.4 ± 0.1
3	20.9	21.3	21.1 ± 0.3
4	27.4	28.4	27.9 ± 0.7
5	33.5	35.6	34.6 ± 1.5
6	40.3	43.2	41.8 ± 2.0

*Leak settings used in the experiment 1 are shown in blue

Pilot experiment 1. Titrating the bacteriophage concentration:

It as was assumed that a patient could emit aerosols with 1.0×10^5 virus copies within a 30-minute period (1). This assumes that the administration of NIV itself does not cause further virus aerosol generation above and beyond what the patient is emitting and noting that patients with seasonal coronavirus have nasal swabs containing, on average, 10^7 virus copies (1). Given that our method of measurement samples only a portion of rooms surface area/viral spread (Total area settling plates = $0.0064m^2$ vs total area room = $13.0m^2$, 0.49%) we performed an experiment designed to assess the concentration of phages in a 10ml sample required to be nebulised in order to be detectable on the plates within the room. To test this 10^5, 10^6, 10^7, 10^8 and 10^9/mL (total 10 mL) concentrations were nebulised for 30 minutes (see Figure S6).

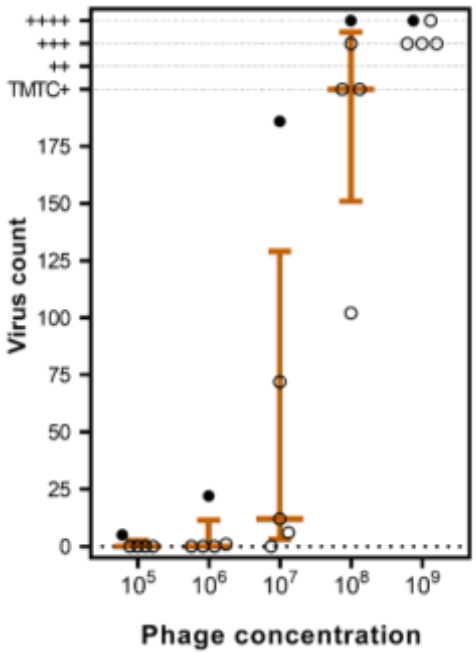


Figure S6. Pilot experiment 1. Plaque count shown according the phage concentration. Orange lines represent median and interquartile range. TMTC = too many to count.

Virus settling was assessed across 5 plates (sites: 1, 3, 5, 9, 13). Plaque counts were mostly zero at concentrations of 10^5, 10^6 and 10^7, excepting the closest plate (5) which reliably demonstrated higher plaque counts across all concentrations. Concentrations of 10^8 and 10^9 showed much high plaque counts on all plates. 10^8/mL (10mls for total dose 10^9) was chosen as the optimal concentration due to having the highest degree of variability in plaque counts across plates.

Pilot experiment 2. Efficacy of HEPA filter to clear room:

After determining the optimal phage concentration, a 10ml mixture was nebulised into the room for 30 minutes. Plates were exchanged in two 15-minute intervals and a final 30-minute interval (see Figure S7A). Plaque count were highest during the nebulisation period (0-30) and remained high for up to 60 mins post nebulisation. This experiment was repeated with the HEPA filter placed on the floor in the middle of the room, set to its highest exchange rate (470 m^3/hr). The filter was turned on immediately after the nebulisation had completed and the initial plates were exchanged (see Figure S7B). Plaque counts rapidly reduced, reaching near zero plaque count on most plates within 15-30 mins. For all subsequent experiments 30 minutes of HEPA filter was used to clear the room between conditions. In all experiments control plates were then positioned for 10 minutes to ensure adequate clearing had occurred.

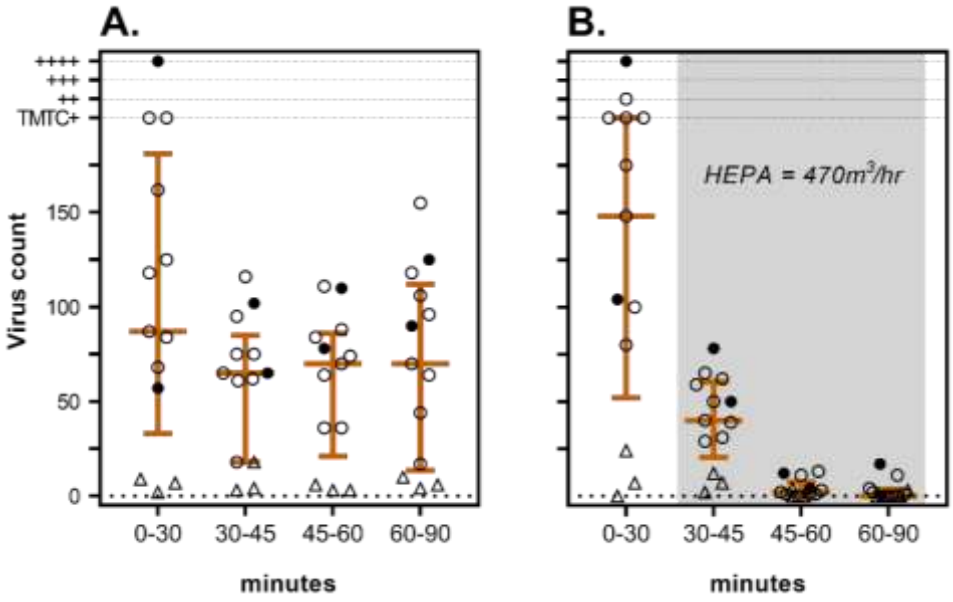


Figure S7. Pilot experiment 2. A. Phages continue to settle for up to 60 mins post-nebulised**.** Note that data shown in the first 3 time points are plotted in the main document in figure 3. **B.** Air purifier set to 470 m^2/hr clears rooms within 30 minutes. Orange lines represent median and interquartile range. TMTC = too many to count.

Relating observed bacteriophage counts to estimated number of SARS-CoV-2 aerosolised virus particles:

Recognising that we are nebulizing 10^8 phages in our experiment but that patients with seasonal coronavirus only generate up to 10^5 aerosolized virus copies we wanted to adjust the observed plaque counts measured in the leak experiments (see main text, Figure 2) to a real-world clinical scenario. In order to do so, we performed the following calculations. The average total number of phage plaques detected for each leak scenario was calculated (sum of the number of plaques per settling plate, divided by three – as each condition was run three times).

Given that only 0.49% of the total surface area of the room was assess with the settling plates, the average number of plaques was multiplied by 204.08 (100 / 0.49**)** to give the total number of plaques we would expect to have detected had the entire surface area of the room been sampled.

Assuming that a hospitalised patient with COVID-19 aerosolises the same number of virus copies as an ambulant/non-hospitalised patient with seasonal coronavirus, and that NIV does not cause additional aerosolised virus to be created (beyond aerosol that the patient generates themselves) then we can multiply the calculated plaque count by a factor that takes into account the total number of phages nebulised in our experiments vs the total number of virus copies a COVID-19 patient can be expected to emit in 30 min. As such, this gives us an estimate of the pure aerosol spread of SARS-CoV-2 from NIV circuit leak. We nebulised 10^9 phages, and Leung et al demonstrated up to 10^5 seasonal coronavirus copies emitted as aerosol (1), indicating that we should adjust our calculated plaque count by 0.0001 (**10^5 / 10^8)**. These calculations are summarised below in Table S3.

Table S3: Adjusted total virus counts

	0 L/min	**7 L/min**	**21 L/min**	**28 L/min**	**42 L/min**
Calculate average total plaques measured on plates:					
$\sum$ (Plates $_{1\text{-}13}$) / 3	83	155	184	281	330
Adjust for reduced fraction of surface air sampling:					
× 204.08 (100/0.49)	17,001	31,681	37,493	57,262	67,353
Adjustment for higher concentration of phages:					
× 0.0001 (10^5 / 10^8)	1.7	3.2	3.8	5.8	6.7
Adjustment for 24 hrs of CPAP usage:					
× 48	82	154	182	278	322
Adjustment for 72 hrs of CPAP usage:					
× 3	245	461	547	835	965

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