ORIGINAL ARTICLE
CRITICAL CARE

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

Shane A. Landry[1], Jeremy J. Barr[2], Martin I. MacDonald[3], Dinesh Subedi[2], Darren Mansfield[3,4,5], Garun S. Hamilton[3,4,5], Bradley A. Edwards[1,6] and Simon A. Joosten[3,4,5]

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

Correspondence: Shane A. Landry, Sleep and Circadian Medicine Laboratory, Ground Floor, Monash University BASE Facility, 264 Ferntree Gully Road, Notting Hill, Victoria 3168, Australia.
E-mail: shane.landry@monash.edu

@ERSpublications
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 a HEPA filter is an efficacious countermeasure. https://bit.ly/2HUiJgn

Cite this article as: Landry SA, Barr JJ, MacDonald MI, *et al*. Viable virus aerosol propagation by positive airway pressure circuit leak and mitigation with a ventilated patient hood. *Eur Respir J* 2021; 57: 2003666 [https://doi.org/10.1183/13993003.03666-2020].

ABSTRACT

Introduction: Nosocomial transmission of severe acute respiratory syndrome coronavirus 2 (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 1) the degree of viable virus propagated from PAP circuit mask leak and 2) the efficacy of a ventilated plastic canopy to mitigate virus propagation.

Methods: Bacteriophage phiX174 (10^8 copies·mL^{-1}) 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^{-1}) was associated with virus counts equivalent to using PAP with a vented mask. The highest frequency of viruses was detected on surfaces <1 m away; however, viable viruses were recorded up to 3.86 m from the source. A plastic hood with HEPA filtration significantly reduced viable viruses on all plates. HEPA exchange rates ⩾170 m^3·h^{-1} eradicated all evidence of virus contamination.

Conclusions: 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.

This article has supplementary material available from erj.ersjournals.com

Received: 29 Sept 2020 | Accepted: 24 Nov 2020

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 PAP (CPAP) or noninvasive ventilation (NIV), is a 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 ("nonvented 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 exist 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 severe acute respiratory syndrome coronavirus 2 (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 3 h and up to 72 h 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 nonclinical 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/"negative pressure rooms", the US Centers 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 (family *Microviridae*), which is a tail-less, icosahedral, nonenveloped, bacteriophage with a linear single-stranded DNA genome. Due to its small size (0.025 µm) 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 airflow exchange rates can mitigate environmental spread of viable virus aerosol.

Methods

A series of experiments was designed to quantify aerosolised viral propagation of a simulated patient with viral respiratory disease (*e.g.* COVID-19) undergoing PAP with a nonvented mask in a nonnegative 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 *Escherichia coli* C (ATCC13706) grown in Tryptic Soy Broth. The lysate was purified following the Phage-on-Tap protocol [21] and resuspended in 1×PBS (Omnipur, Gibbstown, NJ, USA). Phage titre was determined by the soft agar overlay method. To quantify viral spread (*via* plaque forming unit), 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 was 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/nonhospitalised patients [9]. A nebulised 10 mL solution of 10^8 phiX174 virions·mL^{-1} (for an effective total dose of 10^9 phages) provided ideal detection sensitivity. Details of the titration experiments can be found in the supplementary material.

Aerosol generation

Aerosols were generated using a nebuliser (PARI PEP; PARI Respiratory Equipment, Midlothian, VA, USA). Medical air (9 L·min^{-1}) was delivered to the nebuliser *via* tubing connected to a wall mounted flow

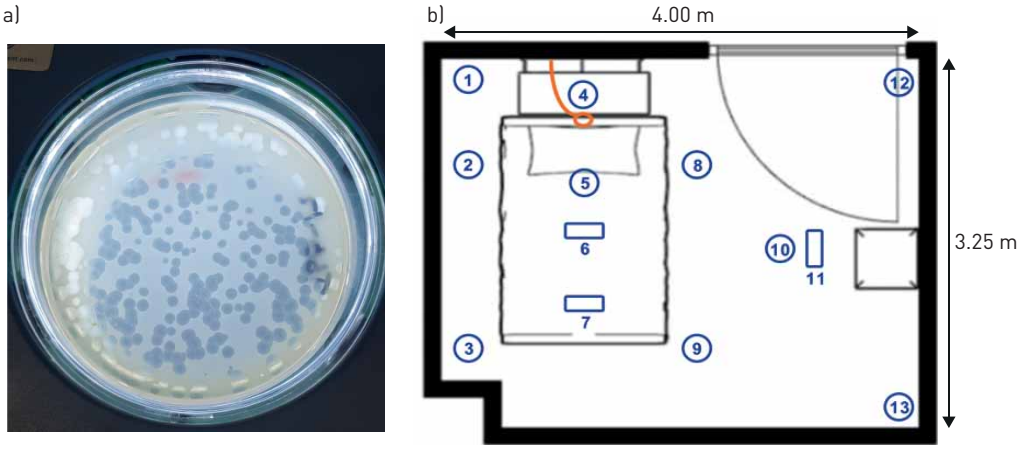

FIGURE 1 Measuring viral dispersion. a) A setting plate with visible plaques. Each plaque indicates a single bacteriophage had settled in that precise location. b) Room layout with locations of the 13 numbered plates shown in blue. Circles represent where plates were positioned on the floor or bed; rectangles show plates hanging from the ceiling oriented perpendicular to the floor. The position of the nebuliser/tubing is indicated in orange.

meter (0–15 $L{\cdot}m^{-1}$, RTM3; Technologie Medicale, Noisy Le Sec, France). 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 three pressure port adapters (900HC452; Fisher & Paykel, Auckland, New Zealand) connected in series. These closable pressure ports (six in 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 (Quattro; ResMed, San Diego, CA, USA) 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 and the second (RT017; Fisher & Paykel) was attached to a viral filter (SureGard, viral filtration efficiency 99.99%, tested against phiX174, RJVKB6; Bird Healthcare, Bayswater, Australia) which served as the filtered expiratory vent. The circuit was attached *via* CPAP hosing (900HC221; Fisher & Paykel) to a pressure source (Pcrit 3000; Phillips Respironics, Murrysville, PA, USA). 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 bilevel (BiPAP) as it can be engineered to provide a continuous and more easily controlled leak profile.

CPAP pressure 15.5 cmH_2O paired with 9 $L{\cdot}min^{-1}$ nebuliser air input produced ~7 $L{\cdot}min^{-1}$ leak increments for each port open such that 0, 7, 21, 28 and 42 $L{\cdot}min^{-1}$ leak could be generated by opening none, one, three, four and six leak ports, respectively. These leak levels were tested and calibrated quantitatively, and were repeatable (supplementary 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 effective dimensions of 4.00×3.25×2.70 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 (mean±SD 21.4±1.0°C) and barometric pressure (mean±SD 758.3±2.5 mmHg) varying minimally during experimental procedures. The room was furnished with a single hospital bed, table and chair.

10 settling plates were placed at specific sites around the room to quantify viruses settling on surfaces and three hanging plates were mounted at head height perpendicular to the floor facing the source (figure 1b). Two settling plates were within 1.0 m of the bedhead/aerosol source, four plates (three settling and one hanging) were within 1.5 m, with the remaining plates being between 1.5 and 3.86 m away (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.

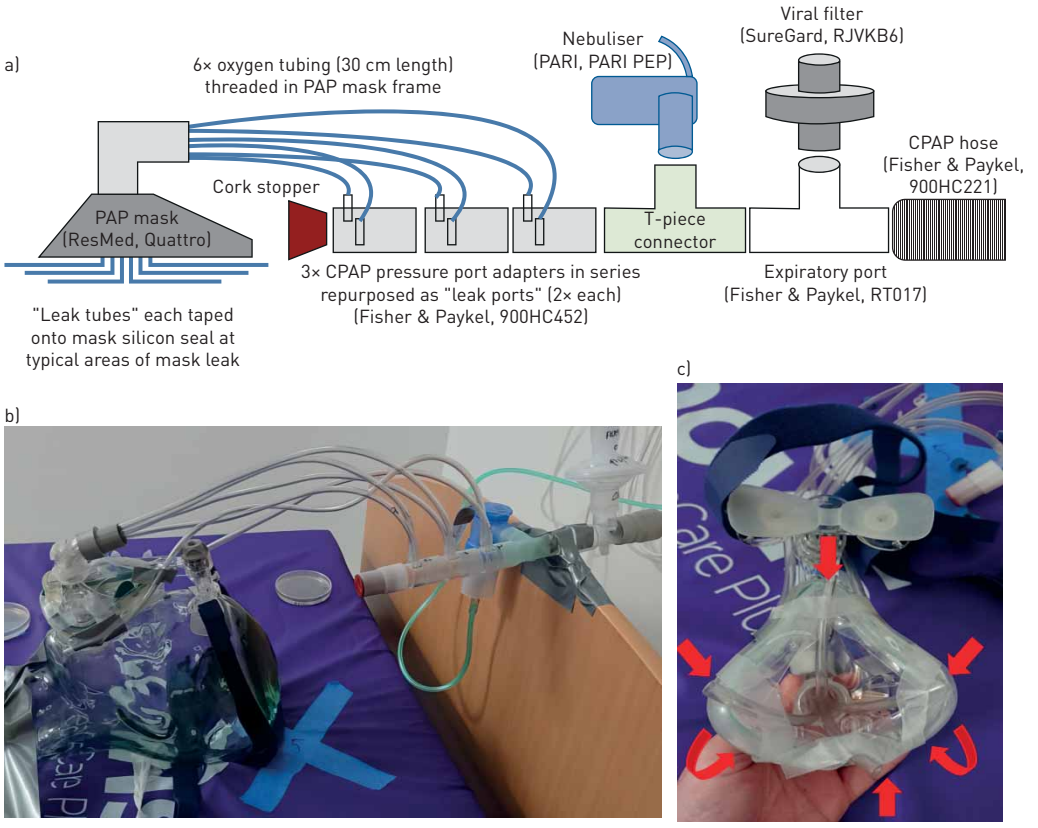

FIGURE 2 Positive airway pressure (PAP) leak circuit. CPAP: continuous PAP. a) Leak circuit diagram. phiX174 bacteriophages are nebulised directly into the pressurised (15.5 cmH_2O CPAP) circuit. Air escapes from the expiratory port (intended leak) which is covered by a viral filter. Leak ports generate ~7 $L \cdot min^{-1}$ leak for each port open. This simulated mask leak is fed *via* tubes into a PAP mask frame. b) Leak circuit (with nebuliser attached). The image shows six leak tubes connected to the mask indicating it is configured to produce leak at 42 $L \cdot min^{-1}$. c) PAP mask with leak tubes. Red arrows show the six locations where air can escape.

HEPA filtration and patient hood structure

An air purifier with HEPA filter (HealthPro 250; IQ Air, Staad, Switzerland) was used to perform multiple air exchanges to clear the room of virus between experiments. Pilot testing (supplementary material) showed that the room could be adequately cleared by 30 min of run time at a flow rate of 470 $m^3 \cdot h^{-1}$ (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 <AUD 40/USD 29). The structure was draped over the top of the bed enclosing the position of 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 min into the leak circuit, which was pressurised at 15.5 cmH_2O. Plates were covered and removed at the end of the 45-min period. This was repeated three times for each leak level (0, 7, 21, 28 and 42 $L \cdot min^{-1}$). 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 $L \cdot min^{-1}$. Between each condition the air purifier was run (at 470 $m^3 \cdot h^{-1}$) for 30 min and then control plates were placed in the room for 10 min 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 min in the room (unconnected to the PAP circuit). Plates were covered and replaced at 30, 45 and 60 min post-nebulisation

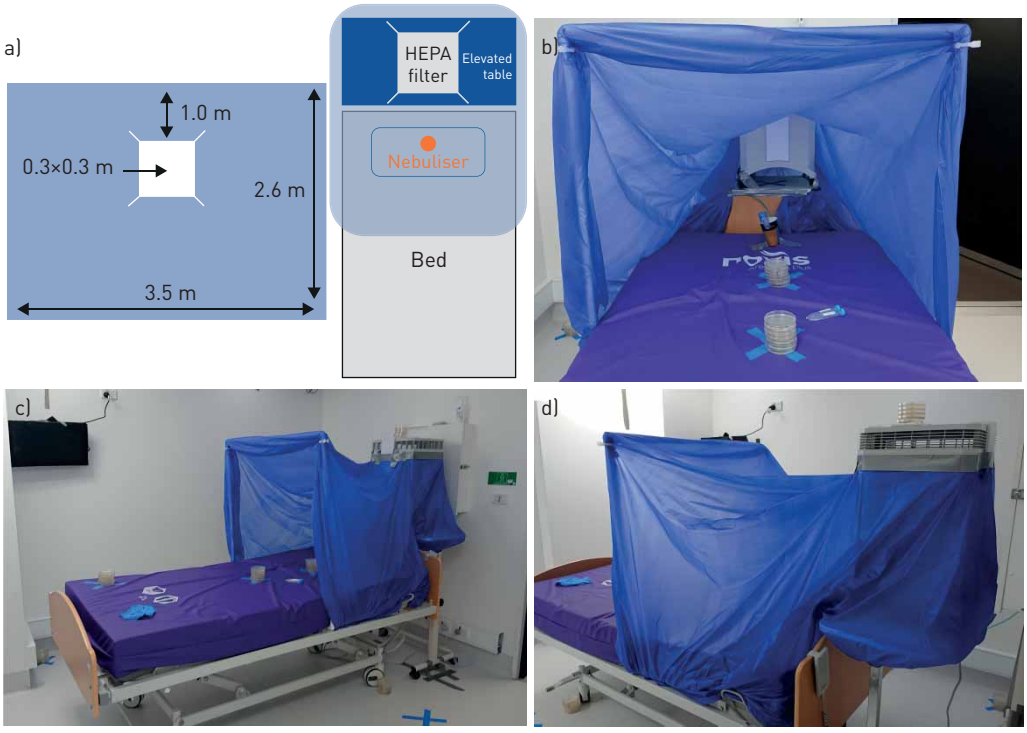

FIGURE 3 Patient hood structure. HEPA: high-efficiency particulate air. a) Hood schematic, and b) front, c) side and d) rear views. The hood structure was constructed out of a tarpaulin sheet with a square-shaped hole (shaped to the size of the HEPA filter) cut in the middle. The hole was then placed over the HEPA filter as a skirt and taped in place. The tail end of the sheet was tucked under the bed mattress and the front end of the sheet was draped over the top part of the bed. A square frame created from PVC piping was placed midway down the bed. The draping sheet 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 the structure was <AUD 40/USD 29.

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 470 $m^3 \cdot h^{-1}$ exchange settings.

Data analysis

In each experiment viable viruses were quantified by counting the number of plaques on settling plates (*i.e.* number of plaque forming units). Plaque counts >200 were considered "too many to count" (TMTC) and were rated using an ordinal visual rating scale (+, ++, +++ and ++++), 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's 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 a nonfiltered, nonsealed circuit (at 0 $L \cdot min^{-1}$ leak) as a reference. Increased leak was associated with an increase in virus counts across settling plates in a dose–response manner (χ^2=58.24, df=4, p<0.001). *Post hoc* tests showed virus counts were significantly lower in the 0 $L \cdot min^{-1}$ condition compared with any other leak level. Similarly, virus counts were higher in the 42 $L \cdot min^{-1}$ condition compared with any other leak level. Mask leak levels ⩾21 $L \cdot min^{-1}$ demonstrated comparable virus counts to when the viral filter was removed from the expiratory vent of the circuit.

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

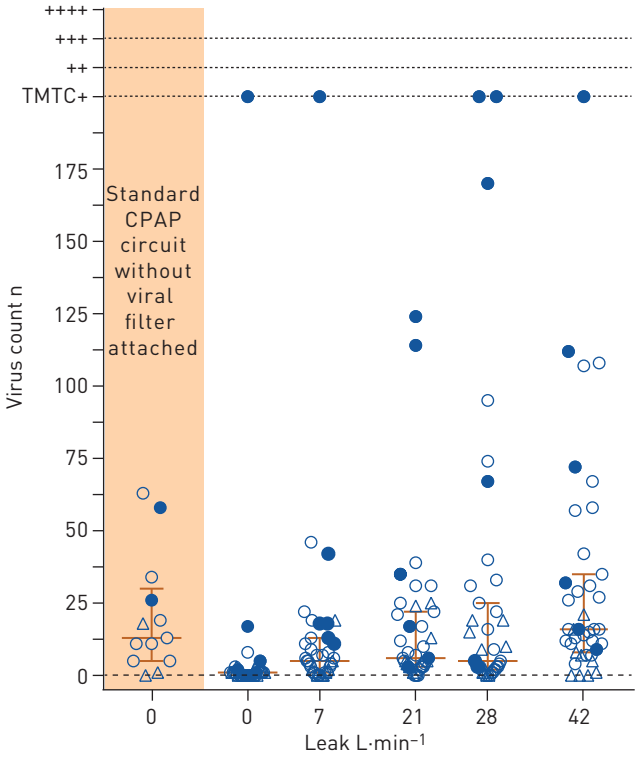

FIGURE 4 Viral aerosol propagation associated with positive airway pressure (PAP) circuit leak. TMTC: too many to count; CPAP: continuous PAP. phiX174 bacteriophages were nebulised over 45 min into in a pressurised (15.5 cmH_2O) PAP circuit designed to leak at either 0, 7, 21, 28 or 42 $L{\cdot}min^{-1}$. As a comparator, the viral filter was removed from the expiratory vent and the circuit set to 0 $L{\cdot}min^{-1}$ leak in order to simulate a nonsealed 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 ⩾21 $L{\cdot}min^{-1}$ spread similar amounts of virus to the environment as an unsealed mask system (a known infection risk factor). Symbols represent virus counts from the 13 individual plates (three replicates for each leak condition, apart from comparator). Filled circles: plates <1 m from the leak source; triangles: hanging plates. Medians and interquartile ranges are indicated. Virus counts >200 were considered "TMTC" and were rated using an ordinal visual rating scale (see Data analysis section).

To assess the relationship with distance, virus counts (combined across all leak conditions, *i.e.* 13 plates×3 replicates×5 leak conditions) were plotted against the distance of each individual plate from the leak source. A shown in figure 5, there was an inverse relationship ($r_{Spearman}=-0.166$, p=0.02, n=195) with virus counts decreasing with distance from source. Notably, the most distant plate (3.86 m) had consistent virus counts at all leak levels ⩾7 $L{\cdot}min^{-1}$.

Efficacy of hood and HEPA filter

When the bacteriophage solution was nebulised directly into the room without the leak apparatus, high virus counts were found on all settle plates, with significant aerosolised viral load persisting up to 60 min after the solution had been completely nebulised (figure 6). Comparatively, hanging plate (positions 6, 7 and 11) virus counts remained relatively low. When viral aerosolisation was repeated with the addition of the hood structure, virus counts were significantly attenuated. The further addition of the HEPA filter had minimal additional efficacy at 50 $m^3{\cdot}h^{-1}$; however, at HEPA settings of 170 and 470 $m^3{\cdot}h^{-1}$ all plates registered zero virus 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 show that aerosols containing viable virus, in similar concentrations to those generated by patients [9], can escape from the PAP system leak and settle onto surfaces at least 3.68 m away from the leak source, even at subclinical levels of leak (7 $L{\cdot}min^{-1}$). 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

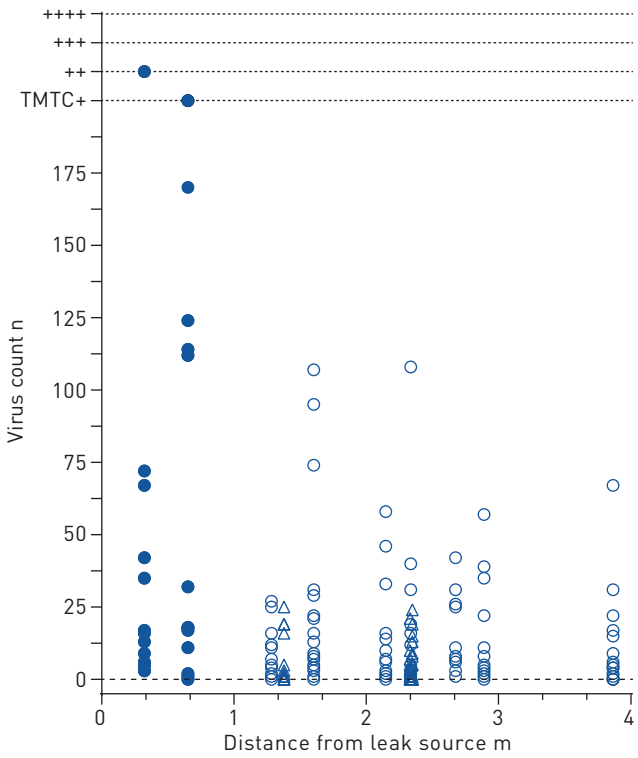

FIGURE 5 Virus counts *versus* distance from leak source. TMTC: too many to count. Symbols represent virus counts from individual plates combined across all leak conditions plotted according to the distance of the plate from the leak source. Specifically, the horizontal and vertical distances of the plate from the leak source were measured and the hypotenuse calculated according to Pythagoras' theorem. Filled circles: plates <1 m from the leak source; triangles: hanging plates. Virus counts >200 were considered "TMTC" and were rated using an ordinal visual rating scale (see Data analysis section).

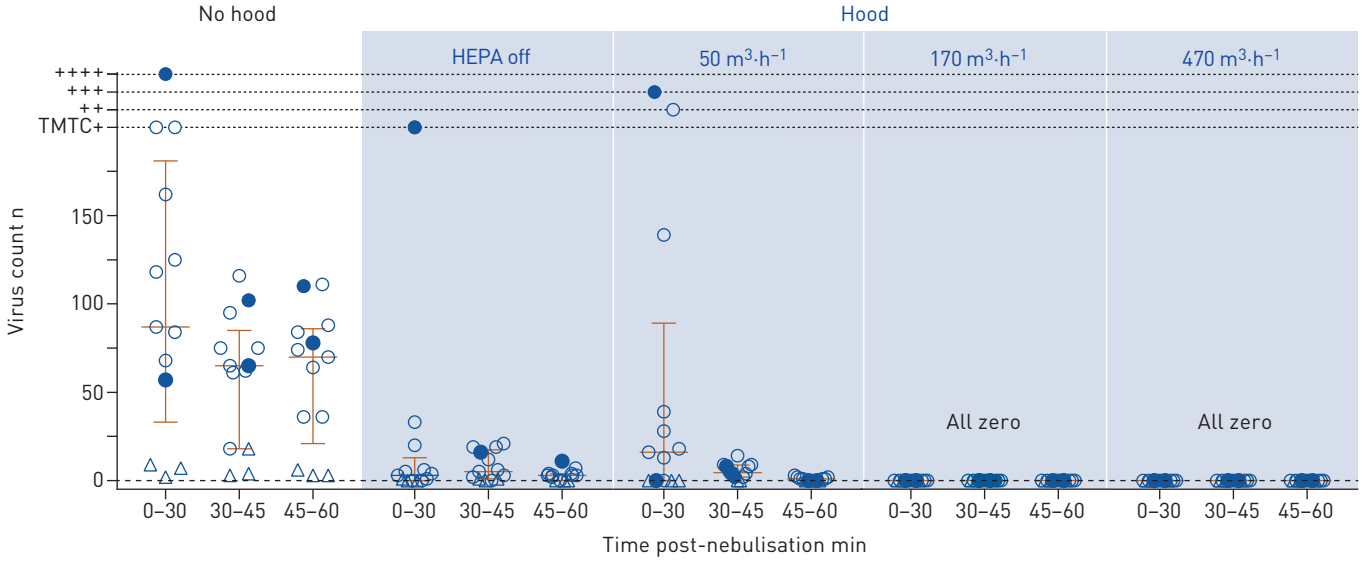

FIGURE 6 The efficacy of the hood and high-efficiency particulate air (HEPA) filter to reduce viral aerosol spread. TMTC: too many to count. phiX174 bacteriophages were nebulised over 30 min. Symbols represent plaque counts from individual plates shown across three time intervals (0–30, 30–45 and 45–60 min post-nebulisation). A hood structure fitted over the head of the bed substantially attenuated virus counts compared with no hood. No plaques were detected on any plates when the HEPA filter was set to 170 or 470 $m^3 \cdot h^{-1}$. Filled circles: plates within the hood (<1 m from nebuliser); triangles: hanging plates. Medians and interquartile ranges are indicated. Virus counts >200 were considered "TMTC" and were rated using an ordinal visual rating scale (see Data analysis section).

sheet and PVC piping, substantially attenuated the degree of virus spreading. Moreover, application of a HEPA filter (to the hood) at an exchange rate $\geqslant$170 $m^3 \cdot h^{-1}$ eliminated all evidence of virus spreading in the environment, even when 10^9 virus copies 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 nonhospitalised patients with seasonal coronavirus are capable of self-generating virus-containing aerosol at rates of 10^5 copies per 30 min, 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 does 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 3 and 72 h. 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 m radius. While techniques that visualise particle spread quantify zones of high-risk/density environmental contamination, expelled aerosols can travel substantially greater distances than 1 m, 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 was shown to impact on plates at head height, and settle on all surfaces, including the most distant point in the room (3.68 m from the source). That virus is detectable at distances >3 m 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 $L \cdot min^{-1}$. 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 noninvasive 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 (30–45 min) of PAP; however, many patients may require substantially longer periods of use (>24 h), which multiplies the associated risk.

The CDC predicted that during a pandemic the 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 ($\geqslant$170 $m^3 \cdot h^{-1}$). 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 aerosol-generating procedures, COVID-19 patients managed in a biocontainment facility with negative pressure at 12 exchanges$\cdot h^{-1}$ exhibited extensive environmental contamination, including in the air and under the beds. An advantage of using our technique compared with a standard negative pressure room is the use of "point of emission" air exchange. A modest air filtration rate for an air purifier (170 $m^3 \cdot h^{-1}$) 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 (~0.025 µm [29]) as SARS-CoV-2 (0.060–0.14 µm). 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 to 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 and NIV).

This study has several limitations. First, we used a nebuliser which produces a tight range of particle size (3.42±0.15 µm) 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 *versus* 10^5 [9]). However, these levels are well balanced by other factors. The settling plates sample ~0.6% of the room's 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 ~10^1). Therefore, we believe that after adjusting for methodological factors which bias towards 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 supplementary table S3. Importantly, with regard to our hood/HEPA experiments, nebulising 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 by 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.

Acknowledgements: The authors would like to thank Christopher Daley and the Monash Cystic Fibrosis Foundation (Clayton, Australia) for generously donating their air purifier for use in this research.

Author contributions: S.A. Landry, J.J. Barr, M.I. MacDonald and S.A. Joosten contributed to study concept and design. S.A. Landry, J.J. Barr, D. Subedi and S.A. Joosten contributed to acquisition of the data. S.A. Landry, J.J. Barr and S.A. Joosten contributed to data analysis. All authors contributed to initial drafting of the manuscript. All authors contributed to interpretation of the data and critical revision of the report.

Conflict of interest: S.A. Landry has nothing to disclose. J.J. Barr has nothing to disclose. M.I. MacDonald has nothing to disclose. D. Subedi has nothing to disclose. D. Mansfield has nothing to disclose. G.S. Hamilton reports nonfinancial support (provision of research equipment) from ResMed, Philips Respironics and Air Liquide Healthcare, outside the submitted work. B.A. Edwards reports grants from Monash Partners and Heart Foundation, during the conduct of the study; grants from Apnimed Australia, personal fees from Signifier Medical, outside the submitted work. S.A. Joosten has nothing to disclose.

Support statement: G.S. Hamilton, D. Mansfield and S.A. Joosten have received equipment to support research from ResMed, Philips Respironics and Air Liquide Healthcare. B.A. Edwards has received funding from Apnimed. B.A. Edwards is supported by a Heart Foundation of Australia Future Leader Fellowship (101167). S.A. Joosten is supported by a National Health and Medical Research Council (NHMRC) Early Career Fellowship (1139745). J.J. Barr is supported by NHMRC New Investigator Grant (1156588).

References

1 Iversen K, Bundgaard H, Hasselbalch RB, *et al.* Risk of COVID-19 in health-care workers in Denmark: an observational cohort study. *Lancet Infect Dis* 2020; 20: 1401–1408.
2 Duca A, Memaj I, Zanardi F, *et al.* Severity of respiratory failure and outcome of patients needing a ventilatory support in the emergency department during Italian novel coronavirus SARS-CoV2 outbreak: preliminary data on the role of helmet CPAP and non-invasive positive pressure ventilation. *EClinicalMedicine* 2020; 24: 100419.
3 Schunemann HJ, Khabsa J, Solo K, *et al.* Ventilation techniques and risk for transmission of coronavirus disease, including COVID-19: a living systematic review of multiple streams of evidence. *Ann Intern Med* 2020; 173: 204–216.

4 Hui DS, Hall SD, Chan MT, *et al.* Noninvasive positive-pressure ventilation: an experimental model to assess air and particle dispersion. *Chest* 2006; 130: 730–740.
5 Somogyi R, Vesely AE, Azami T, *et al.* Dispersal of respiratory droplets with open vs closed oxygen delivery masks: implications for the transmission of severe acute respiratory syndrome. *Chest* 2004; 125: 1155–1157.
6 Tran K, Cimon K, Severn M, *et al.* Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. *PLoS One* 2012; 7: e35797.
7 Raboud J, Shigayeva A, McGeer A, *et al.* Risk factors for SARS transmission from patients requiring intubation: a multicentre investigation in Toronto, Canada. *PLoS One* 2010; 5: e10717.
8 Fowler RA, Guest CB, Lapinsky SE, *et al.* Transmission of severe acute respiratory syndrome during intubation and mechanical ventilation. *Am J Respir Crit Care Med* 2004; 169: 1198–1202.
9 Leung NHL, Chu DKW, Shiu EYC, *et al.* Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med* 2020; 26: 676–680.
10 van Doremalen N, Bushmaker T, Morris DH, *et al.* Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020; 382: 1564–1567.
11 Santarpia JL, Rivera DN, Herrera VL, *et al.* Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci Rep* 2020; 10: 12732.
12 Feng S, Shen C, Xia N, *et al.* Rational use of face masks in the COVID-19 pandemic. *Lancet Respir Med* 2020; 8: 434–436.
13 Ye G, Lin H, Chen S, *et al.* Environmental contamination of SARS-CoV-2 in healthcare premises. *J Infect* 2020; 81: e1–e5.
14 Sanche S, Lin YT, Xu C, *et al.* High contagiousness and rapid spread of severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis* 2020; 26: 1470–1477.
15 Karagiannidis C, Mostert C, Hentschker C, *et al.* Case characteristics, resource use, and outcomes of 10 021 patients with COVID-19 admitted to 920 German hospitals: an observational study. *Lancet Respir Med* 2020; 8: 853–862.
16 Nguyen LH, Drew DA, Graham MS, *et al.* Risk of COVID-19 among front-line health-care workers and the general community: a prospective cohort study. *Lancet Public Health* 2020; 5: e475–e483.
17 Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet* 2020; 395: 1225–1228.
18 National Institute for Occupational Safety and Health. Engineering controls to reduce airborne, droplet and contact exposures during epidemic/pandemic response: ventilated headboard. 2020. www.cdc.gov/niosh/topics/healthcare/engcontrolsolutions/ventilated-headboard.html Date last accessed: December 4, 2020.
19 Verreault D, Moineau S, Duchaine C. Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev* 2008; 72: 413–444.
20 Turgeon N, Toulouse M-J, Martel B, *et al.* Comparison of five bacteriophages as models for viral aerosol studies. *Appl Environ Microbiol* 2014; 80: 4242–4250.
21 Bonilla N, Rojas MI, Netto Flores Cruz G, *et al.* Phage on tap – a quick and efficient protocol for the preparation of bacteriophage laboratory stocks. *PeerJ* 2016; 4: e2261.
22 Berlinski A. *In vitro* evaluation of positive expiratory pressure devices attached to nebulizers. *Respir Care* 2014; 59: 216–222.
23 Shen Z, Ning F, Zhou W, *et al.* Superspreading SARS events, Beijing, 2003. *Emerg Infect Dis* 2004; 10: 256–260.
24 Kim S, Jeong YD, Byun JH, *et al.* Evaluation of COVID-19 epidemic outbreak caused by temporal contact-increase in South Korea. *Int J Infect Dis* 2020; 96: 454–457.
25 Hui DS, Chow BK, Ng SS, *et al.* Exhaled air dispersion distances during noninvasive ventilation *via* different Respironics face masks. *Chest* 2009; 136: 998–1005.
26 Mead KR, Feng A, Hammond D, *et al.* Expedient methods for surge airborne isolation within healthcare settings during response to a natural or manmade epidemic. 2012. www.cdc.gov/niosh/surveyreports/pdfs/301-05f.pdf Date last accessed: December 4, 2020.
27 Johnson DL, Lynch RA, Mead KR. Containment effectiveness of expedient patient isolation units. *Am J Infect Control* 2009; 37: 94–100.
28 McGain F, Humphries RS, Lee JH, *et al.* Aerosol generation related to respiratory interventions and the effectiveness of a personal ventilation hood. *Crit Care Resusc* 2020; 22: 212–220.
29 Yazaki K. Electron microscopic studies of bacteriophage phi X174 intact and 'eclipsing' particles, and the genome by the staining, and shadowing method. *J Virol Methods* 1981; 2: 159–167.
30 Johnson GR, Morawska L, Ristovski ZD, *et al.* Modality of human expired aerosol size distributions. *J Aerosol Sci* 2011; 42: 839–851.