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.
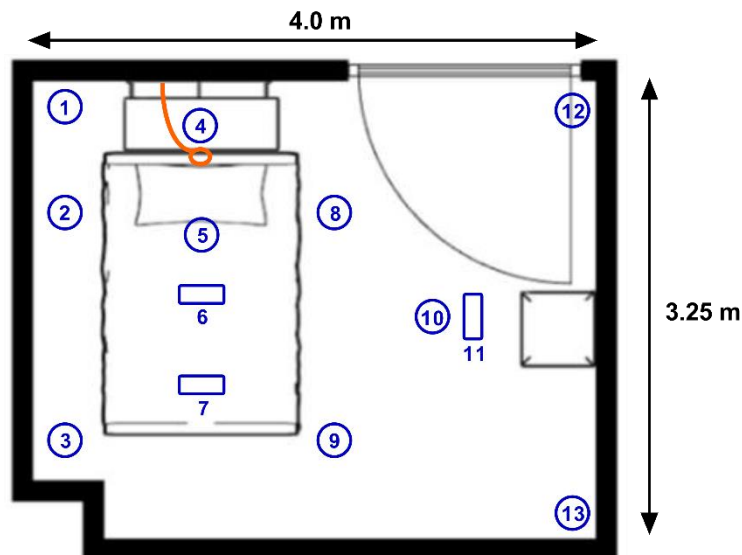
*Figure S1: Room layout and plate locations.*

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	Y distance (cm) 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.
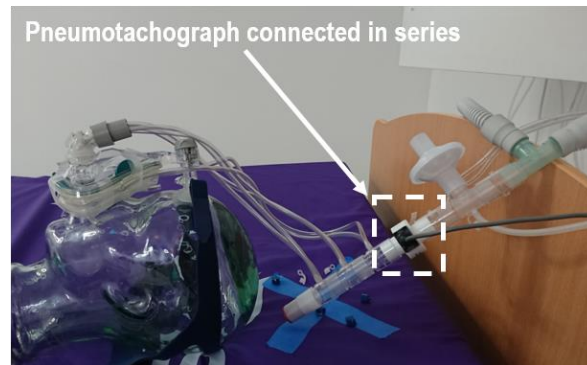
Figure S4. Leak settings validated using Pneumotachograph.

A CPAP level of 15.5 cmH$_2$O 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.

| Number of ports open | Leak (L/min) | | |
	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).
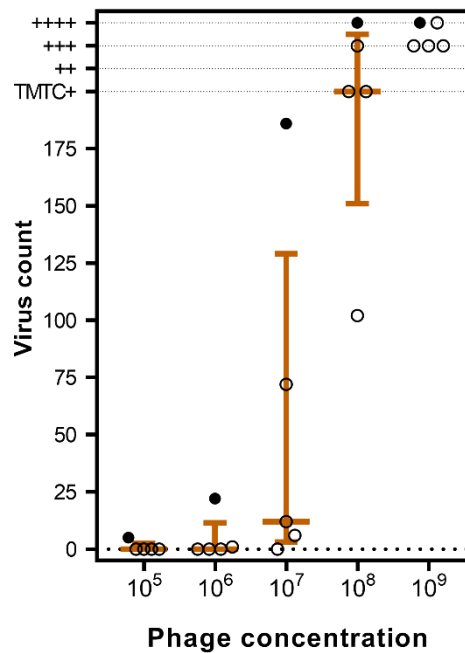

Figure S6. Pilot experiment 1. Plaque count shown according the phage concentration. Orange lines represent median and interquartile range. TMTC = too many to count.

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.
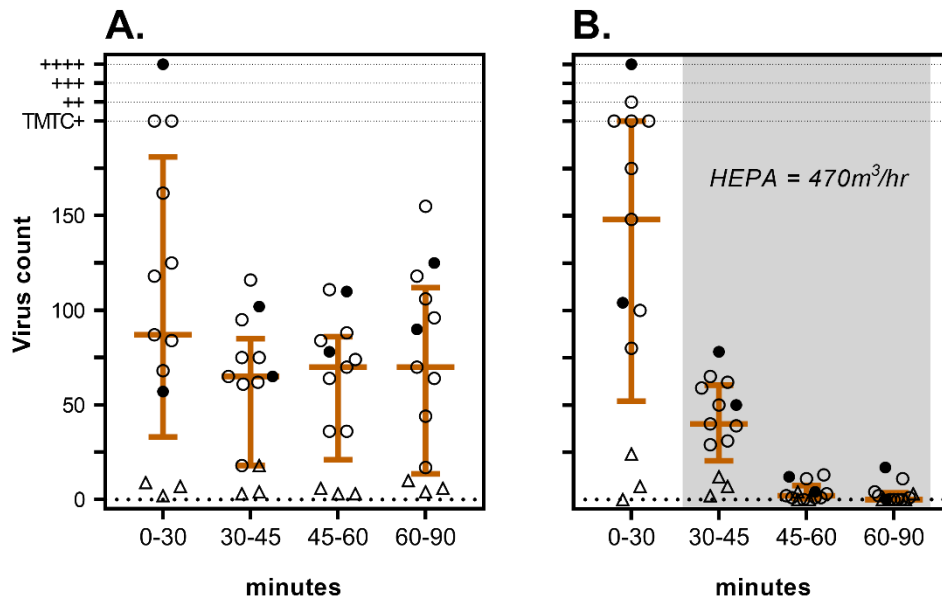
Figure S7. Pilot experiment 2. A. Phages continue to settle for up to 60 mins post-nebulised**.** Note that data shown in the first 3 time points are plotted in the main document in figure 3. **B.** Air purifier set to 470 m^2/hr clears rooms within 30 minutes. Orange lines represent median and interquartile 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 $_{1-13}$**) / **3**	83	155	184	281	330
Adjust for reduced fraction of surface air sampling:					
$\times$ **204.08 (100/0.49)**	17,001	31,681	37,493	57,262	67,353
Adjustment for higher concentration of phages:					
$\times$ **0.0001 (10^5 / 10^8)**	1.7	3.2	3.8	5.8	6.7
Adjustment for 24 hrs of CPAP usage:					
$\times$ **48**	82	154	182	278	322
Adjustment for 72 hrs of CPAP usage:					
$\times$ **3**	245	461	547	835	965

REFERENCES

1. Leung NHL, Chu DKW, Shiu EYC, Chan KH, McDevitt JJ, Hau BJP, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat Med. 2020;26(5):676-80.