ONLINE SUPPLEMENTARY MATERIAL

VIABILITY OF *PSEUDOMONAS AERUGINOSA* IN COUGH AEROSOLS GENERATED BY PERSONS WITH CYSTIC FIBROSIS.

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METHODS

Recruitment

Subjects with CF were recruited from the two CF centres by personal invitation from the Investigators (SCB, CEW) or the Study Coordinators (MEW, JC). All CF patients were >12-years of age, had chronic *Pseudomonas aeruginosa* infection and at least one positive sputum culture in the prior 12-months. The majority of patients were studied during clinical stability, however, four patients were studied close to the completion of intravenous antibiotics for logistic reasons (e.g. travelling long distance, avoid missing school attendance). Healthy control participants were recruited from contact with staff at both healthcare facilities including relatives of the staff for the younger controls. Nineteen CF patients with CF and 10 healthy controls participated. Two patients and two controls performed only one of the experiment days.

Distance of cough aerosols: 'Distance Rig'

The Distance Rig consisted of an expandable wind tunnel that allowed high-efficiency particulate air (HEPA)-filtered air to be introduced upstream of the subject;[1] ensuring unidirectional air flow of particle-free air and permitting measurement of cough aerosols at distances of 0 to 4-metres from the subject without interference from other particle sources (Figure E1). The air velocity was maintained at 0.1-metres/sec, typical of a mechanically-ventilated indoor environment, such as a hospital.[2-4] The Rig was positively pressurized to prevent room air contamination.

Viable aerosol and total particle sampling methods

A six-stage Andersen Impactor (Thermo Scientific, Franklin, MA) captured and sized viable cough aerosols in the six stages between 0.6 and >7 μm.[5] A vacuum pump was used to

draw 28.3 L/min of air through the Impactor. The pump flow was checked daily using a rotameter, and all Andersen Impactor O-rings were inspected for wear. Exhaust air from the pump was HEPA-filtered.

A Lasair II-110 (Particle Measuring Systems, Boulder, CO) optical particle counter (OPC) measured real-time particle concentration in six channels between 0.1 and >5 µm using a sample flow of 28.3 L/min, permitting detection of very low concentrations. The OPC verified that there were no room air particles present before each test, subjects' lungs were free of residual room air, and no contamination of aerosols occurred during testing. It also confirmed that the total particle concentration in the Rig was <0.01 particles per cm^3 (p/cc) prior to the subject coughing, which was approximately 10^4 times lower than the room air concentration and similar to an ISO 4 cleanroom.

The Andersen Impactor and OPC samples were collected through a common 12.5 cm circular, sharp-edged isokinetic inlet. It was set parallel to the airflow and provided 100% particle aspiration efficiency at the tunnel air velocity of 0.1 metres/sec.[6] Smoke visualization tests confirmed sample extraction by the inlet was uniform. One metre of conductive tubing transported samples from the inlet to the Andersen Impactor and OPC. The tubing residence time was 1-sec for Andersen samples and 0.06-sec for OPC samples. Sample losses due to gravitational settling and inertial impaction were from 0 to 8% and 0 to 1%, respectively, and diffusion losses were negligible.[6]

Air velocity was monitored continuously using a 9535 hot-wire anemometer (TSI Inc., St. Paul, MN). Temperature and water vapour concentration were measured simultaneously

upstream of the participant and at the sampling inlet by HC2-CO4 probes and a HygroLog NT data logger (Rotronic AG, Bassersdorf).

Duration of cough aerosols: 'Duration Rig'

A 0.4 $metre^3$ airtight stainless steel cylinder was used for collecting and aging cough aerosols to assess viability in the airborne phase (Figure E2). A variable-speed drive was used to set the rotation rate (1.7 rpm) of the Rig, which minimized gravitational settling and inertial impaction of particles.[7, 8] The system was flushed with HEPA-filtered air before sample collection so that cough aerosols were prevented from being contaminated by room air aerosols.

Viable aerosol and total particle sampling methods

The Andersen Impactor and OPC described previously were used during sampling. We estimate over 95% of cough aerosol particles in the size range of the Andersen Impactor remained airborne during the aging period.[9]

An 8-cm circular inlet was positioned 30-cm inside the Rig along the rotation axis. This provided 100% aspiration efficiency of particles in the Andersen and OPC size ranges.[6] Samples were transported via straight conductive tubing, with residence times of 0.8 sec for Andersen samples and 0.09 sec for OPC samples. Sample losses across the Andersen Impactor size range due to gravitational settling and inertial impaction were from 0 to 6% and 0 to 1%, respectively. Temperature and water vapour concentration were measured simultaneously inside and outside of the Duration Rig by the same probes described above.

Before each test, the Duration Rig was flushed with HEPA-filtered air using a 3M Air-Mate respirator (St. Paul, MN). Once the OPC confirmed a particle concentration <0.01 p/cc, a 5-minute blank sample was taken by the Andersen Impactor. The Air-Mate provided filtered air to replace that extracted by the pump. The OPC confirmed the Rig was free of contamination, and the Rig was then isolated by entry and exit valves.

Cleaning and quality assurance

The Distance and Duration Rigs were disinfected thoroughly at the end of each study day using 0.15% (w/v) benzalkonium chloride (Glitz™, Pascoe's Pty Ltd, Australia), followed by 70% (v/v) ethanol. HEPA-filtered air dried both devices. Non-disposable items, collection tubes, inlet valves and other rigging devices were sterilized using standard hospital procedures. Andersen Impactors were decontaminated with 70% (v/v) ethanol and air dried. Surface swabs were collected into Amies Agar Gel Transport Medium (COPAN Diagnostics Inc., CA, USA) and blank aerosol samples were collected at the start and end of each day. For Duration studies, additional blank aerosol samples were collected between each experiment.

Quality assurance surface swabs were enriched in LB broth (Sigma-Aldrich Pty Ltd, New South Wales, Australia) for 24-hours, and then subcultured onto chocolate bacitracin and colistin nalidixic acid agar (Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) at 35^0C for 72 hours, and Sabouraud agar (Thermo Fisher Scientific Australia Pty Ltd) at 28^0C for 72 hours. Blank aerosol samples were incubated aerobically at 35^0C for 72-hours.

Modelling of airborne *Pseudomonas aeruginosa*

To estimate the time taken to remove airborne *P. aeruginosa* following the departure of a source patient, we used a simple model based on the airborne biological inactivation rate we measured experimentally combined with a range of room ventilation rates.[10] This approach assumed that these were the two major mechanisms by which airborne *P. aeruginosa* was removed; biological inactivation (i.e. 'die-off') and dilution with room ventilation air.[10] Our modelling focussed solely on the risk posed by airborne cough aerosol droplet nuclei containing *P. aeruginosa*, as distinct from contact or droplet transmission, as this is the transmission mode affected by these removal processes.

The role of airborne biological decay and room air ventilation was considered to be negligible in determining the fate of particles in the size range collected by the first (i.e. 'input') stage of the viable sampler. This input stage collects particles >7μm and such droplet nuclei were not considered to fall within the airborne size range (Dp <5μm) for the purposes of this study. The decision to exclude the larger droplet nuclei is further supported by the expectation that droplet nuclei form initially as much larger droplets before drying to their equilibrium size and collection by the viable sampler. That initial size is approximately twice the diameter of the droplet residue collected by the viable sampler.[11] The smaller surface area to mass ratios of these larger particles results in the droplets' movement and fate being dominated by momentum acquired within the cough exhalation jet and the influence of gravity immediately after the cough. Because of these influences the larger droplets and droplet residues tend to impact on surfaces during, or soon after, the cough event so that they do not remain airborne long enough to have their fate altered significantly by the ventilation and room air currents.[12-14] Hence, deposition of particles is not incorporated into airborne models. While we observed small numbers of viable particles on the input stage of the viable sampler

following extended storage, this was mostly due to the counteraction of the gravitational settling process provided by the Duration Rig.[9] Thus, under ‘real world’ conditions, particles in the size range of the Andersen Impactor input stage would deposit due to gravitational settling very shortly after their release, in contrast to those in the airborne range, which remain suspended for extended periods.[14] We therefore considered the particles collected on the input stage to not be relevant to our modelling.

Empirically, the effect of ventilation on the concentration of airborne pathogens, or indeed any particle, over time follows a first-order exponential decay.[11, 15] Likewise, the biological inactivation of a pathogen in response to environmental challenges is represented in the same manner.[11] Therefore, the concentration of *P. aeruginosa* at a given point in time can be calculated using equation 1:

$$\log_e(N_t) = \log_e(N_0) - (k_{bio} + k_{vent}) \times t \qquad (1)$$[1]

Where:

N_t = $P. aeruginosa$ concentration at time t (CFU L^{-1})

N_0 = $P. aeruginosa$ concentration at time 0 (CFU L^{-1})

k_{bio} = biological inactivation rate of $P. aeruginosa$ (h^{-1})

k_{vent} = removal of $P. aeruginosa$ due to room ventilation rate (ACH^{-1})

t = time (h)

A practical example of when to employ such models is when seeking to adhere to the US Centers for Disease Control and Prevention recommendation of allowing sufficient time for ≥99% removal of airborne contaminants following the departure of a patient with suspected or confirmed tuberculosis from a room before another patient enters.[16]

Similarly, the model can help determine the time required to achieve a specified reduction in airborne *P. aeruginosa* in a CF clinic setting by taking into account the known room ventilation rate. Figure 3 in the paper shows that it would take approximately 50-min to achieve this removal, due to combined effect of ventilation and biological inactivation, in a clinic room ventilated at the guideline rate of two ACH.[17-19] Figure 3 also shows the time taken to remove specified amounts of *P. aeruginosa* at the ventilation guidelines prescribed for other clinical settings.[17]

In addition to assuming that ventilation and biological decay are the major mechanisms which remove airborne *P. aeruginosa* from room air, we made two other key assumptions that underpin equation 1 and its use that affect the accuracy of our predictions. Firstly, and most importantly, the pathogen is assumed to be to be perfectly mixed with the room air, which is almost never the case in practice.[17] There are regions where the concentration can be higher or lower than that assumed under perfect mixing. For example, this could occur due to proximity of the pathogen source or a room ventilation inlet, respectively. However, this assumption is characteristic of all the classic airborne transmission models.[17] If a conservative approach to infection control is required, then it is appropriate to err towards a greater amount of pathogen removal, such as 90%, to account for this when determining how long to wait before the next person enters a room.

Secondly, as the majority of ventilation air is usually recirculated it is prudent to base estimates of room clearance on only the outdoor air component of ventilation air, which is the method we have used to present the estimates in this paper.[20] For example, if 25% of air supplied to a room is 'fresh' outdoor air and the total ventilation rate is 8 ACH, then the outdoor air exchange rate is 2 ACH and this is the appropriate figure to use when modelling

or reading off Figure 3 in the paper. Likewise, ventilation guidelines for healthcare settings often specify a minimum total ventilation rate and the proportion that must be outdoor air (e.g. 6 ACH total with at least 2 ACH outdoor). In assessing a room ventilated at these guideline values, the ventilation rate would be set to 2 ACH, rather than 6. This approach considers all recirculated ventilation air as contaminated and incapable of diluting the concentration of a pathogen. While this may not be the case in practice, particularly where recirculated air is filtered, it allows for conservative exposure risks to be determined. This method has its basis in the enduring work of Riley and colleagues in revisiting the earlier work of Wells, and developing what is now known as the Wells-Riley equation for estimating the probability of airborne pathogen transmission indoors.[21, 22] As the infectious inoculum of *P. aeruginosa* is unknown, it is appropriate to employ this conservative approach to infection control.

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Table S1. Repeatability of log transformed combined total colony-forming units (CFU) of *Pseudomonas aeruginosa*, total number of coughs, FEV1 actual and $\log_{10}$ sputum *P. aeruginosa* in subjects with cystic fibrosis.

	Experiment	Repeatability	95% CI
Combined total	Distance	0.92	0.86 - 0.97
	Duration	0.89	0.79 - 0.96
Total number of coughs	Distance	0.95	0.90 - 0.98
	Duration	0.95	0.91 - 0.98
FEV_1 actual		0.79	0.48 - 0.92
$\log_{10}$ sputum *P. aeruginosa*		0.71	0.35 - 0.89

Table S2. Comparison of between subjects, within subjects, duplicates and ratios for log transformed small, large and combined total particle fraction colony-forming units (CFU) of *Pseudomonas aeruginosa* isolated from the cough aerosol cultures of subjects with cystic fibrosis and the number of coughs per minute in distance and duration experiments.

Characteristic	Between subjects	Within subjects	Duplicates	Between/Within Ratio	Between/Duplicates Ratio
Distance					
Small	21.5	0.6	0.2	38.1	99.4
Large	21.8	0.5	0.2	44.7	113.9
Combined total	24.6	0.5	0.3	46.5	112.8
Number of coughs	1245.6	20.6	6.6	60.6	189.9
Duration					
Small	16.8	0. 6	0.3	32.9	56.6
Large	9.8	0.5	0.2	22.3	47.1
Combined total	17.8	0.6	0.3	31.9	69.4
Number of coughs	1523.3	14.8	20.5	100.6	74.3

Table S3. Colony-forming unit (CFU) counts of *Pseudomonas aeruginosa*, corrected for stacking, isolated from the cough aerosol cultures of subjects with cystic fibrosis. CFUs in total aerosol, and in large and small particle fractions are shown*.

Distance	1 metre	2 metres	4 metres	ANOVA P-value†
Total‡	59.3 (45.6 – 77.0)[a]	39.4 (30.2-51.3)[b]	26.3 (20.1-34.3)[c]	0.001
Large fraction§	30.2 (23.4-38.9)[a]	21.0 (16.2-27.1)[b]	13.2 (10.1-17.2)[c]	<0.001
Small fraction‖	31.7 (24.0-41.7)[a]	20.8 (15.6-27.5)[b]	15.3 (11.5-20.3)[b]	0.003
Duration	5 minutes	15 minutes	45 minutes	
Total‡	15.2 (11.4-20.1)[a]	12.3 (9.2-16.4)[a]	7.9 (5.5-11.3)[b]	0.043
Large fraction§	4.3 (3.2-5.7)[a]	4.0 (2.9-5.3)[a]	2.3 (1.5-3.4)[a]	0.073
Small fraction‖	12.5 (9.5-16.5)[a]	9.3 (7.0-12.3)[ab]	6.4 (4.4-9.1)[b]	0.029

* Values are means (95% CI). Within sets and rows a different superscript letter ([a] or [b] or [c]) denotes a significant difference (P<0.05) between other distances or durations.

† ANOVA for trend across distance or duration

‡ Total CFU counts represent the *P. aeruginosa* CFUs isolated from all six Andersen Impactor Stages (aerosol particles sizes 0.65 to >7.0 µm).

§ Large particle fraction CFU counts represent the *P. aeruginosa* CFUs isolated from Stages 1, 2 and 3 (aerosol particle sizes >7, 4.7-7.0 and 3.3-4.7 µm, respectively) of the Andersen Impactor.

‖ Small particle fraction CFU counts represent the *P. aeruginosa* CFUs isolated from Stages 4, 5 and 6 (aerosol particle sizes 2.1-3.3, 1.1-2.1 and 0.65-1.1 µm, respectively) of the Andersen Impactor.

Table S4. Correlation between clinical characteristics, number of coughs performed, sputum *Pseudomonas aeruginosa* concentration and log transformed combined total colony-forming units of *P. aeruginosa* isolated from the cough aerosol cultures of subjects with cystic fibrosis.

Characteristic	Pearson correlation coefficient					
	1 metre	2 metres	4 metres	5 minutes	15 minutes	45 minutes
Age	-0.05	-0.2	-0.25	0.02	-0.02	-0.11
Body-mass index	-0.37	-0.46	-0.36	-0.16	-0.19	-0.24
FEV_1 Value - litres	0.08	0.22	0.21	-0.12	0.08	0.06
FEV_1 Percent of predicted value	0.09	0.25	0.22	0.02	0.24	0.23
FVC Value - litres	0.05	0.13	0.16	-0.22	-0.07	-0.11
FVC Percent of predicted value	0.07	0.2	0.2	0	0.18	0.17
MIP Value - cmH2O	-0.1	-0.14	-0.16	-0.29	-0.33	-0.36
MIP Percent of predicted value	-0.31	-0.26	-0.28	-0.34	-0.35	-0.27
MEP Value - cmH2O	0.27	0.29	0.22	0.12	0.14	0.05
MEP Percent of predicted value	-0.15	-0.02	-0.09	-0.22	-0.12	-0.06
Number of coughs performed	-0.05	0.04	-0.09	0.23	0.26	0.26
Sputum *P. aeruginosa* concentration	0.73*	0.73*	0.78†	0.90†	0.85†	0.85†

* Correlations achieving statistical significance of 0.01.

† Correlations achieving statistical significance of <0.01.

Table S5. Correlation between clinical characteristics, number of coughs performed, sputum *Pseudomonas aeruginosa* concentration and log transformed combined total colony-forming units of *P. aeruginosa* corrected for stacking isolated from the cough aerosol cultures of subjects with cystic fibrosis.

Characteristic	Pearson correlation coefficient					
	1 metre	2 metres	4 metres	5 minutes	15 minutes	45 minutes
Age	-0.06	-0.20	-0.26	0.02	-0.02	-0.10
Body-mass index	-0.37	-0.46	-0.36	-0.16	-0.19	-0.23
FEV_1 Value - litres	0.08	0.22	0.21	-0.12	0.07	0.06
FEV_1 Percent of predicted value	0.10	0.25	0.22	0.03	0.23	0.22
FVC Value - litres	0.05	0.13	0.16	-0.22	-0.08	-0.12
FVC Percent of predicted value	0.08	0.20	0.21	0.00	0.17	0.16
MIP Value - cmH2O	-0.10	-0.14	-0.16	-0.29	-0.33	-0.36
MIP Percent of predicted value	-0.28	-0.26	-0.26	-0.33	-0.35	-0.27
MEP Value - cmH2O	0.24	0.27	0.20	0.12	0.14	0.05
MEP Percent of predicted value	-0.13	-0.01	-0.08	-0.21	-0.12	-0.06
Number of coughs performed	-0.05	0.04	-0.09	0.24	0.26	0.26
Sputum *P. aeruginosa* concentration	0.71*	0.73*	0.77†	0.90†	0.85†	0.85†

* Correlations achieving statistical significance of 0.01.

† Correlations achieving statistical significance of <0.01.

Figure S1. Schematic diagram of the Distance Rig.

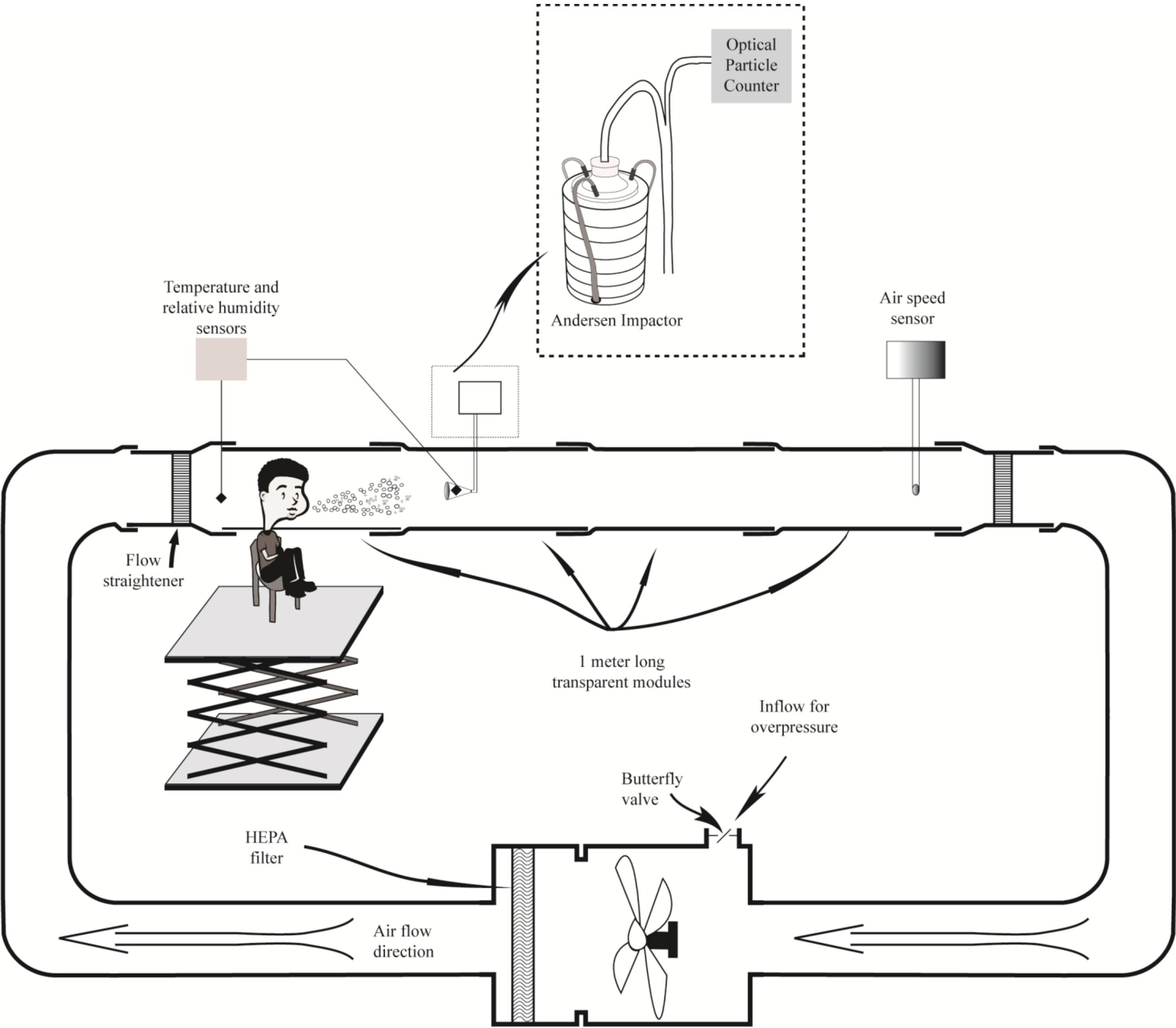

Figure S2. Schematic diagram of the Duration Rig.