SUPPORTING INFORMATION

The susceptibility of an airborne common cold virus to relative humidity

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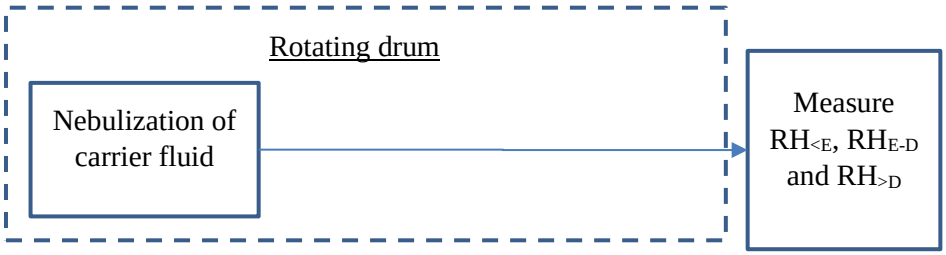
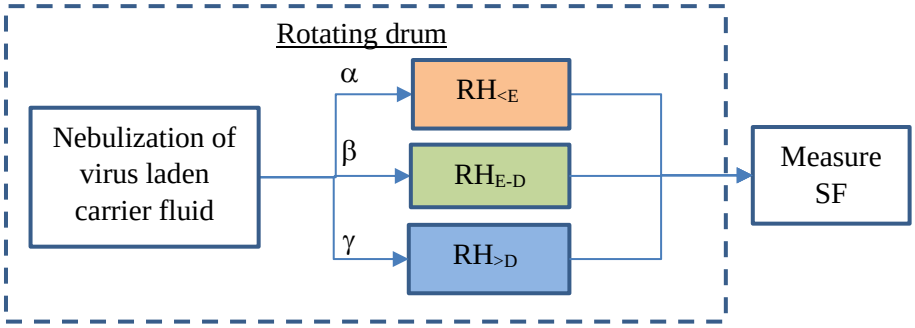
Phase 1- Hygroscopic characterization (locating the hysteresis zone)



Phase 2 - Virus decay in the $RH_{<E}$, RH_{E-D} and $RH_{>D}$ zones without Pre-treatment



Phase 3 - Virus decay in RH_{E-D} zone in the Effloresced and Deliquesced (Control) States.

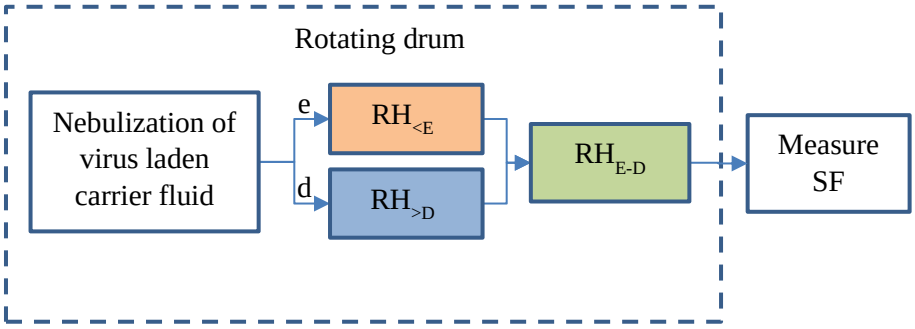


Figure S1: Experimental process. Labels "α, β and γ" denote phase 2 experiments. Labels "e" and "d" denote phase 3 experiments.

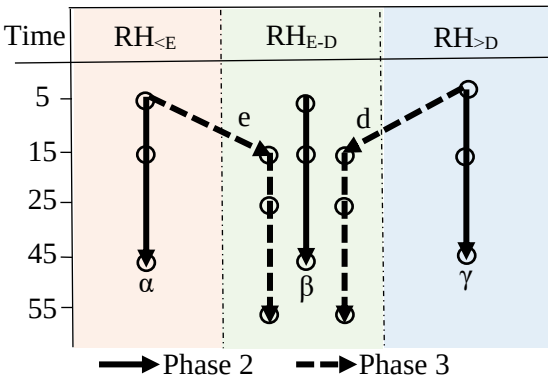


Figure S2: Sample timing. Sample ages at the times of extraction are indicated by the symbol "O". Phase 2 samples are linked by continuous lines labelled "α, β and γ". Phase 3 by dashed lines labelled "e" and "d". Samples were collected and SF determined at aerosol age timepoints of 5, 15 and 45 minutes for phase 2 and 5, 15, 25 and 55 minutes for phase 3.

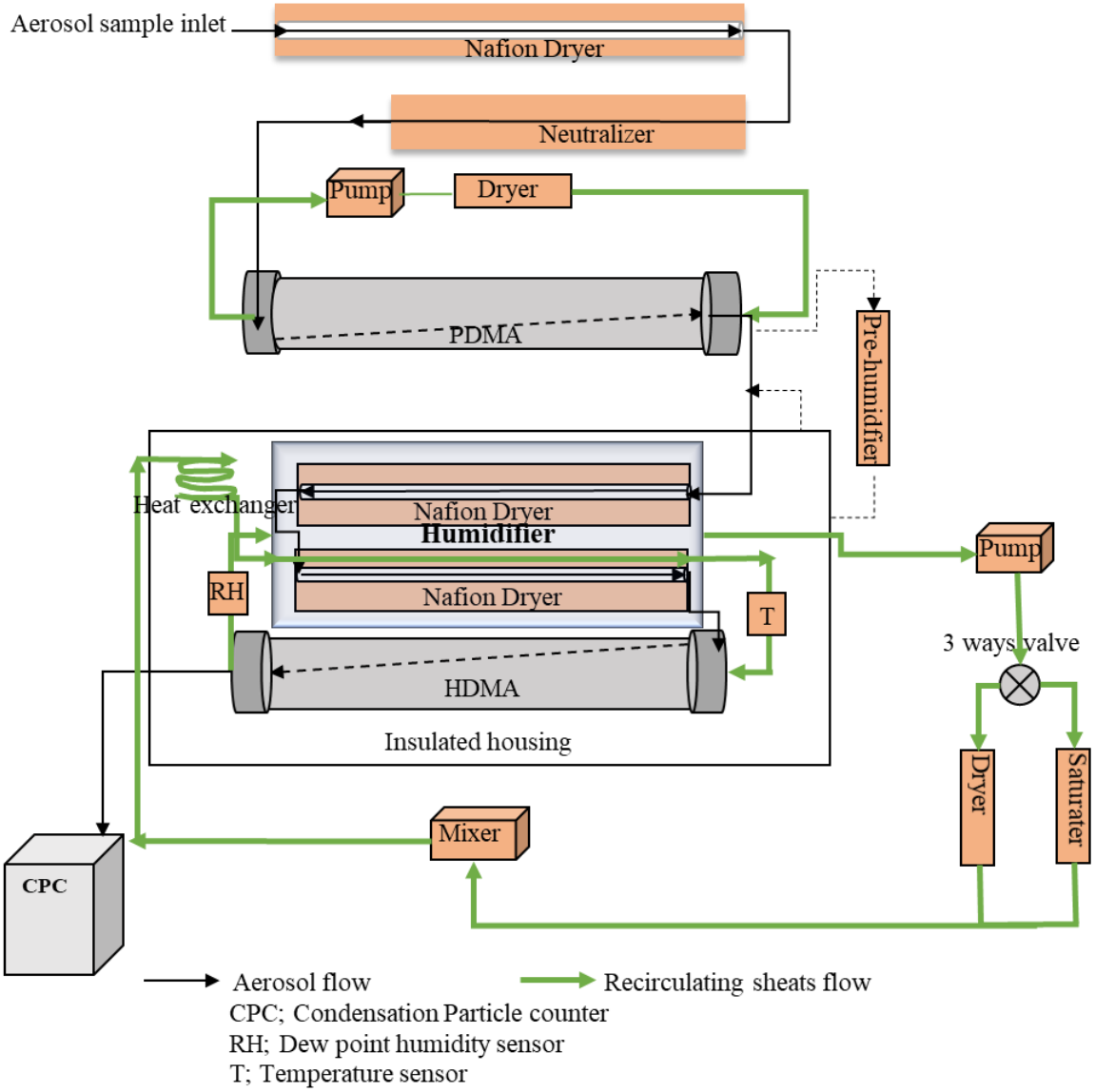


Figure S3: Schematic diagram of the applied H-TDMA instruments.

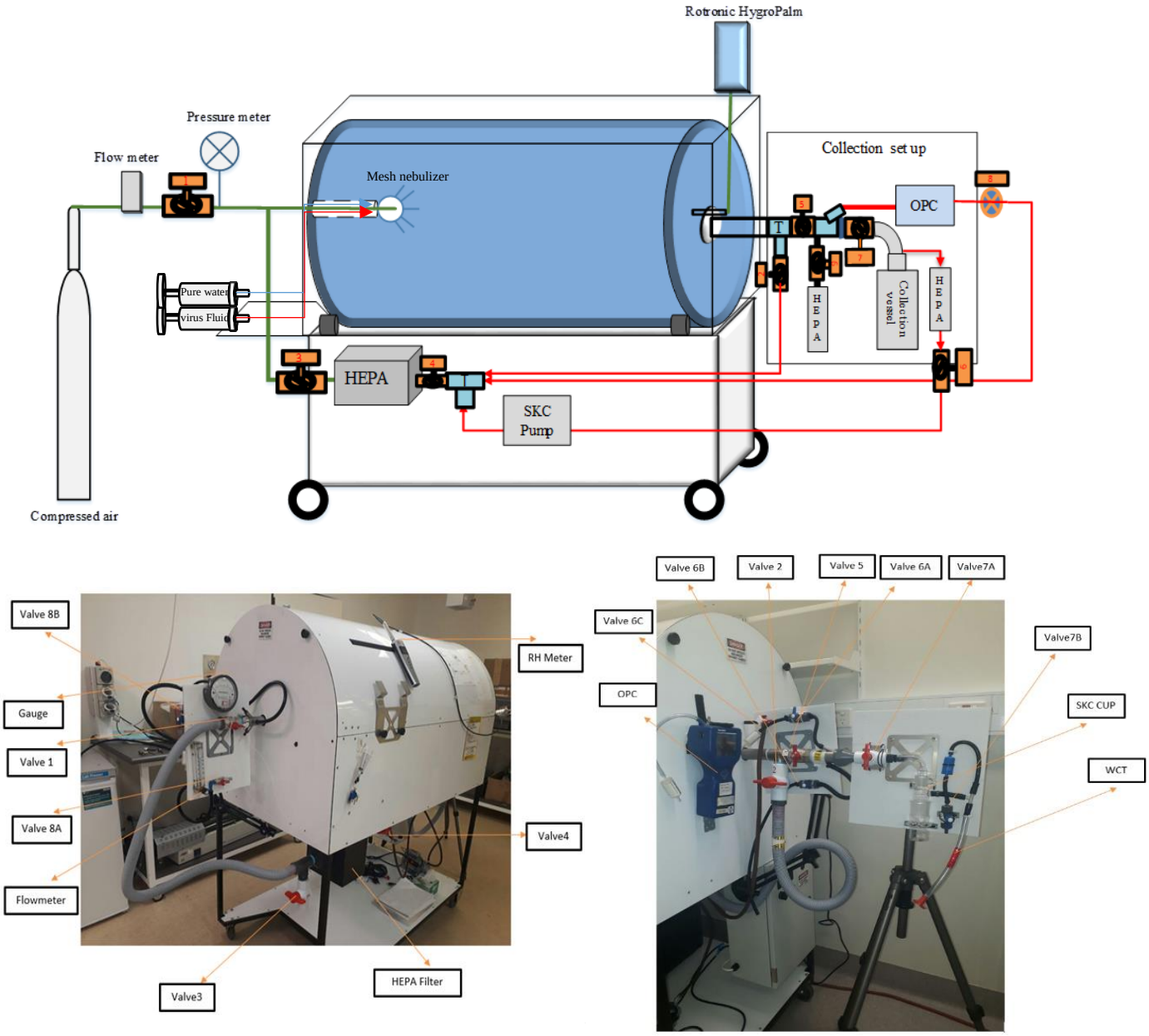


Figure S4: Schematic and photograph of the rotating chamber and instrument interfaces (rotator, valves and connectors). WCT; water collection tube, OPC; Optical Particle Counters.

Text I

Temperature control was achieved using a thermoelectric heating and cooling system embedded in the wall of the outer enclosure and controlled by a proportional integral derivative (PID) feedback system connected to a temperature sensor inside the outer enclosure. This allowed for internal temperature adjustment within ±0.5 °C, for the range 13-30 °C. A Rotronic HygroPalm - HP23-A with 1 minute time interval was used to measure RH and temperature inside the drum. For measuring the virus survival in an effloresced state, virus suspension was first nebulized inside the system over a period of 5 minutes, starting from a very low RH (6%). The water component of the nebulized aerosol increased the RH to 33% (still less than the efflorescence RH measured for the aerosol). A small amount of ultrapure nuclease free water purified by membrane filtration was then nebulized over a period of 5 minutes to bring the system into the hysteresis RH range at approximately 57% of RH. To test virus survival in a deliquesced (droplet) state, water was first nebulized to bring the system to 80% and the virus suspension was then nebulized over a period of 5 minutes, bringing the system up to 90.4% RH, which is well above the deliquescence RH measured for the aerosol. Dry air was then injected over a period of 5 minutes after nebulization to reduce the RH back to a hysteresis range value of 57%.

Text II

The novel use of vibrating mesh nebulizer was chosen over the more traditional Collison nebulizer in the current application because it offered a very high fluid nebulization rate (1.5 mL/min) without the need for a carrier air flow, allowing better temporal resolution in time sensitive measurements. The nebulizer employs an electroformed plate perforated with conical holes and mounted on a piezoelectric ceramic ring that causes the plate to vibrate in response to an applied alternating potential difference. The vibrating mesh draws fluid through the holes to generate aerosols without the need for a carrier flow [1]. The plate used in the nebulizer was 5 mm in diameter, pierced with 1000 tapered holes and vibrated at a frequency of 128 kHz to produce a unimodal droplet size distribution with diameters between 1 and 5 μm in diameter [2].

Text III

The aerosols were extracted from the drum using an SKC Biosampler with 5 ml collection vessels (#225-9593, SKC). The BioSampler is an improved impinger-type air sampler consisted of a 5 ml collection vessel, connecting tube and a pump with the flow rate of 12.5 L min^{-1} and collects aerosols using a swirling flow of liquid media (PBS by drawing air through three 0.630 mm tangential sonic nozzles [3].

Text IV

HRV-16 was grown in Ohio HeLa cells in VP-SFM (Life Technologies, USA). Once 80% cell death was achieved, cell culture supernatant and remaining cells were collected and centrifuged at 2000 ×g to form cell pellets. Supernatant was removed and the pellets were freeze-thawed twice to disrupt cell membranes and release bound viral particles, then clarified by centrifugation at 2000 × g. This clarified preparation and the original cell supernatant were combined, then the virus was concentrated by centrifugation through 15 mL spin columns (size cut-off 0.2µm) (Merc Millipore Amicon Ultra) at 2500 × g for 25min at 4°C. The concentrated virus was quantified using a tissue culture infectious dose ($TCID_{50}$) assay, and stored at -80 °C.

Table S1: The amount of extracted RNA (ng/ml) and $TCID_{50}$/mL for each experiment at $RH_{<E}$, RH_{E-D} and $RH_{>D}$.

experiment	Aging time	$RH_{<E}$		RH_{E-D}		$RH_{>D}$	
		RNA (ng/ml)	Measured $TCID_{50}$	RNA (ng/ml)	$TCID_{50}$ /ml	RNA (ng/ml)	$TCID_{50}$ /ml
1	Before	37657.14	12500000	42000	93700000	38685.82	52500000
	5 minutes	3085.71	167000	3045.72	395000	2754.28	1250000
	15 minutes	2685.73	125000	3005.71	395000	2634.34	527000
	45 minutes	2257.14	52700	2257.16	225000	2474.29	39500
2	Before	39371.42	22200000	42457.14	93700000	38857.15	52500000
	5 minutes	2828.57	525000	2811.42	703000	2800	1250000
	15 minutes	2822.85	395000	2800.16	395000	2817.14	937000
	45 minutes	2514.28	296000	2725.71	12500	2702.86	125000
3	Before	28514.28	2960000	42405.73	93700000	39028.57	52500000
	5 minutes	2514.28	52500	2788.58	395000	3668.57	1250000
	15 minutes	2628.57	39500	2668.57	125000	3280	937000
	45 minutes	2457.14	29600	2657.14	62700	2702.85	39500

Table S2: The amount of extracted RNA (ng/ml) and $TCID_{50}$/mL for each experiment of effloresced (treatment case) and deliquesced (control-treatment case) states.

experiment	Aging time	Effloresced treatment case		Deliquesced (control-treatment) case	
		RNA (ng/ml)	Measured $TCID_{50}$	RNA (ng/ml)	Measured $TCID_{50}$
1	Before	26651.43	703000	36742.81	22200000
	15 minutes	3040	12500	1942.85	395
	25 minutes	2994.26	9370	2114.28	222
	55 minutes	2990	5270	1428.57	16
2	Before	44400	29600000	36742.8	22200000
	15 minutes	3617.14	325000	1650	10395
	25 minutes	2914.28	125000	1500	3937
	55 minutes	2691.42	7030	1465	3076
3	Before	44400	52500000	36742.81	22200000
	15 minutes	3251.43	837000	1885.71	3600
	25 minutes	2622.86	527000	834.22	1500
	55 minutes	2680	125000	1428.51	800

Table S3: One-way ANOVA results of surviving fraction at different aging times for $RH_{<E}$, RH_{E-D} and $RH_{>D}$ (* 0.033, ** 0.002 and ***0.001).

Tukey's multiple comparisons test of SFs between sampling times.	Mean Diff.	Significant?	Summary	Adjusted P Value
5min $RH_{<E}$ versus 5min RH_{E-D}	0.15	Yes	*	0.03
5min $RH_{<E}$ versus 5min $RH_{>D}$	-0.07	No	ns	0.7
5min RH_{E-D} versus 5 min $RH_{>D}$	-0.22	Yes	***	<0.001
15min $RH_{<E}$ versus 15min RH_{E-D}	0.12	No	ns	0.1
15min $RH_{<E}$ versus 15min $RH_{>D}$	-0.02	No	ns	>0.999
15min RH_{E-D} versus 15min $RH_{>D}$	-0.15	Yes	*	0.03
45min $RH_{<E}$ versus 45min RH_{E-D}	0.11	No	ns	0.2
45min $RH_{<E}$ versus 45min $RH_{>D}$	0.11	No	ns	0.2
45min RH_{E-D} versus 45min $RH_{>D}$	0.0001	No	ns	>0.999
5 min $RH_{<E}$ versus 15 min $RH_{<E}$	0.053	No	ns	0.9
5min $RH_{<E}$ versus 45min $RH_{<E}$	0.099	No	ns	0.3
15min $RH_{<E}$ versus 45min $RH_{<E}$	0.045	No	ns	0.9
5min RH_{E-D} versus 15 min RH_{E-D}	0.026	No	ns	0.9
5min RH_{E-D} versus 45 min RH_{E-D}	0.051	No	ns	0.9
15 min RH_{E-D} versus 45 min RH_{E-D}	0.03	No	ns	0.9
5min $RH_{>D}$ versus 15 min $RH_{>D}$	0.1	No	ns	0.3
5min $RH_{>D}$ versus 45 min $RH_{>D}$	0.3	Yes	***	<0.001
15min RH_{D} versus 45 min $RH_{>D}$	0.2	Yes	**	0.008

Table S4: One-way ANOVA results of surviving fraction at different aging times for Effloresced (treatment case) and deliquesced (control-treatment case) states (* 0.033, ** 0.002 and ***0.001).

Tukey's multiple comparisons test	Mean Diff.	Significant?	Summary	Adjusted P Value
Surviving fraction of 15 minutes effloresced state versus 15 minutes deliquesced state	0.16	Yes	***	<0.001
Surviving fraction of 25 minutes effloresced state versus 25minutes deliquesced state	0.11	Yes	***	<0.001
Surviving fraction of 55 minutes effloresced case versus 55 minutes deliquesced state	0.03	No	ns	0.72
Surviving fraction of 15 minutes versus 25 minutes effloresced state	0.05	No	ns	0.36
Surviving fraction of 15 minutes versus 55 minutes effloresced state	0.13	Yes	**	0.002
Surviving fraction of 15 minutes versus 25 minutes deliquesced state	0.002	No	ns	0.999
Surviving fraction of 15 minutes versus 55 minutes deliquesced state	0.003	No	ns	>0.999

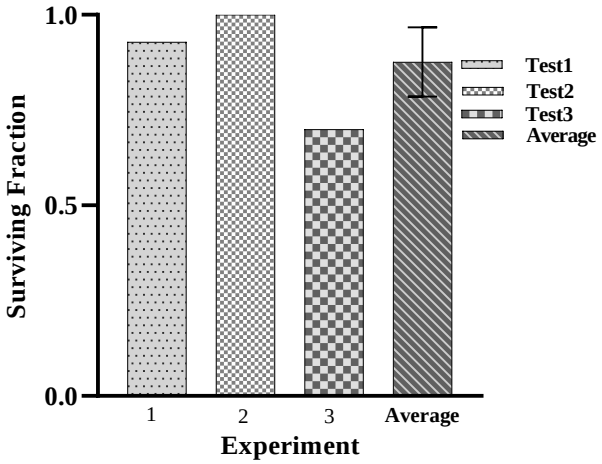


Figure S5: HRV-16 survival from vibrating mesh nebulizer testing before and after aerosolization (Results represent three experimental replicates and their average). Mean Surviving Fraction (SF) = 0.87±0.15.

REFERENCES

(1) Ghazanfari, T.; Elhissi, A. M.; Ding, Z.; Taylor, K. M., The influence of fluid physicochemical properties on vibrating-mesh nebulization. *Int J Pharm* **2007,** *339*, 103-11.

(2) Astudillo, A.; Leung, S. S. Y.; Kutter, E.; Morales, S.; Chan, H. K., Nebulization effects on structural stability of bacteriophage PEV 44. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **2018,** *125*, 124-130.

(3) Willeke, K.; Lin, X.; Grinshpun, S. A., Improved aerosol collection by combined impaction and centrifugal motion. *Aerosol Science and Technology* **1998,** *28*, 439-456.