Supplemental Table 1. Average log decay for H1N1pdm09 or Spn in droplets

		Log_{10} Decay H1N1pdm09 ± SEM		Log_{10} Decay Spn ± SEM	
ASL Donor	HBE Donor Condition	H1N1pdm09	H1N1pdm09/Spn	Spn	H1N1pdm09/Spn
0223	COPD	2.333 ± 0.068*	1.667 ± 0.068*	3.187 ± 0.067*	2.565 ± 0.002*
0259	COPD	1.083 ± 0.136	1.125 ± 0.118	3.071 ± 0.101*	2.577 ± 0.033*
0284	IPF	0.958 ± 0.180	1.292 ± 0.180	4.950 ± 0.415*	2.848 ± 0.148*
0305	COPD	1.00 ± 0.118	0.708 ± 0.272	4.237 ± 0.333	3.256 ± 0

An asterisk indicates the p-value was less than 0.05 when comparing droplets of individual microbes to droplets with both microbes using a Welch's unpaired t-tests.

Supplemental Materials and Methods

Virus and Bacteria

A/California/07/2009 (H1N1pdm09) was grown in minimum essential media in Madin-Darby Canine Kidney (MDCK) cells at 37°C for 48 hours and collected by centrifuging supernatant to remove cell debris. Quantification of virus was performed using the 50% tissue culture infectious dose assay ($TCID_{50}$) of 10-fold serial dilution on MDKC cells in 96-well plates and subsequent assessment for cytopathic effects 4 days after plating.

S. pneumoniae D39 (Spn) was grown in Columbia broth at 37°C. Quantification of bacterial burden was performed by plating 10-fold serial dilutions on blood agar plates and counting colony-forming units after incubation at 37°C overnight.

Animals

Experiments involving ferrets were performed at the University of Pittsburgh under BSL2 safety conditions (IACUC protocol 19075697). Four to six-month male ferrets were confirmed to be seronegative for influenza infection prior to purchase. Animals were intranasally infected with 10^6 $TCID_{50}$ of H1N1pdm09 in 500 µL total volume and 10^7 CFU of Spn in 500 µL. Ferrets were sedated using isoflurane prior to nasal wash collection, performed by collecting the flow-through of PBS passed through the nostrils.

Air Sampling

Infectious virus and bacteria were collected using the Liquid Spot Sampler (Aerosol Devices Inc, Series 110), which uses condensation to collect aerosols into a collection vial. Air was collected from infected animals in a 7 liter chamber connected to the Spot sampler via anti-static tubing for

15 minutes each day at a rate of 1.4L/minute (Supplemental Figure 1). Sampling was performed on days 3, 4, and 5 post-H1N1pdm09 infection (days 1, 2, and 3 post-Spn infection) and prior to nasal wash collection. Condensed aerosols were collected in 700µL 0.5% BSA in PBS. Samples were immediately plated to quantify expelled bacteria and the remaining sample was used for virus titration as described above.

Aerosol sampling of H1N1pdm09/Spn-infected ferrets was performed using cyclone-based air samplers (BC251 developed by NIOSH) on days 3, 4, and 5 post-H1N1pdm09 infection to collect microbial genomic material. Samplers, calibrated to collect 3.5L of air per minute, were placed downwind of infected animals in cages with directional airflow and were run for 1 hour. Samplers fractionated aerosols into three sizes: aerosols >4µm, 1-4µm, and <1µm diameter. After aerosol collection, samplers were washed with isopropanol and allowed to air-dry to avoid contamination.

RNA was isolated using 500µL MagMAX Lysis/Binding Solution Concentrate in each collection tube with thorough vortexing. QIAamp viral RNA mini kit was used to isolate DNA and RNA from lysis solution. Viral and bacterial genome copies were quantified using RT-qPCR with primers against influenza M gene (Forward 5'-AGATGAGTCTTCTAACCGAGGTCG-3' ; Reverse 5'-GCAAAGACACTTTCCAGTCTCTG-3' ; Probe 5'-[FAM]TCAGGCCCCCTCAAAGCCGA[3BHQ1] -3') or *S. pneumoniae* lytA gene (Forward 5'-ACGCAATCTAGCAGATGAAGCA-3' ; Reverse 5'-TCGTGCGTTTTAATTCCAGCT-3' ; Probe 5'-[HEX]GCCGAAAACGCTTGATACAGGGAG[BHQ1]-3'). *In vitro* transcribed RNA was used to make a standard curve for influenza virus, and *S. pneumoniae* genomic DNA was serially diluted to generate a standard curve for Spn. Limits of detection were determined by a Ct = 40 or a positive day 0 sample.

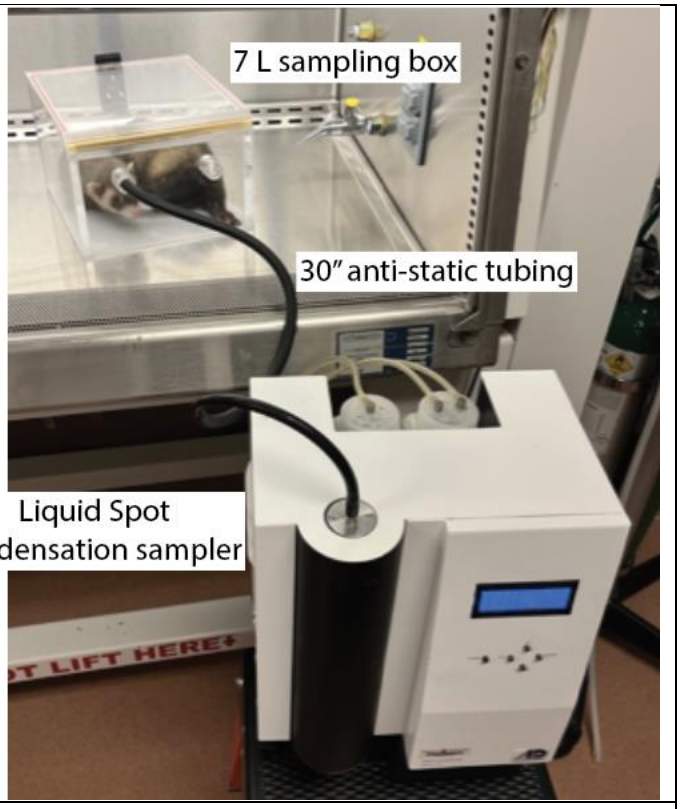
252 Stability Experiments

253 Inside a biosafety cabinet, a saturated salt solution of K_2CO_3 was used to condition a glass
254 chamber to 43% relative humidity, and a HOBO UX-100-011 logger was used to record
255 temperature and humidity conditions during each ASL replicate (Figure 2E). Experimental
256 solutions were generated using $10^{7.15}$ CFU/mL Spn, $10^{7.15}$ $TCID_{50}$/mL H1N1pdm09, and a 1:5
257 dilution in PBS of airway surface liquid collected from human bronchial epithelial cells. Ten 1
258 µL droplets were incubated on polystyrene tissue culture plates in the conditioned chamber for
259 two hours. Controls were 10 µL samples of each microbial solution in closed tubes that were
260 incubated for 2 hours at ambient temperature during the chamber experiments. Log_{10} decay was
261 calculated as previously described and represents the loss in virus or bacterial infectivity (21).
262 Log_{10} decay was determined for each droplet replicate by subtracting the titer of the droplets
263 from the average of the controls for the corresponding ASL. Experiments were performed using
264 technical triplicates for droplets and technical duplicates for controls.

265 Human lung tissue collected using an approved protocol was used to differentiate human
266 bronchial epithelial cells as previously described (22). Airway surface liquid was collected by
267 washing differentiated cells with 150 µL PBS and collecting the wash (3). All HBE donors were
268 diagnosed with chronic obstructive pulmonary disease (COPD), except for HBE 0284, which
269 came from a patient diagnosed with idiopathic pulmonary fibrosis.

270 **Data Availability**

271 The data that supports the findings shown here will be made openly available in FigShare at
272 DOI: 10.6084/m9.figshare.22129055 upon publication. Some of the stability experiments were
273 previously made available on BioRxiv at https://doi.org/10.1101/2020.11.10.376442



Supplemental Figure 1. The Liquid Spot condensation sampler was used to collect infectious material from co-infected animals. Co-infected animals were placed in a sampling box for 15 minutes while sampling was performed. Anti-static tubing connected the sampling box to the inlet of the sampler.

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