Supporting Information

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SI Materials and Methods

NP swabs were eluted in 1 mL of elution medium consisting of either PBS supplemented with 0.1% BSA (PBS/0.1% BSA) or universal transport medium (Copan). Teflon impactors were scrubbed using a nylon flocked swab (Copan) saturated with PBS/0.1% BSA. The end of the swab was cut off and placed in a tube containing 1 mL PBS/0.1% BSA. The tube was vortexed for 1 min at full speed to elute material from the swab, and the swab head was removed. Fine-aerosol buffer samples were concentrated to 1 mL using a CentriconPlus-70 (EMD Millipore) centrifugal ultrafiltration device with a nominal molecular mass cut-off of 100 kDa. Samples were stored either at 4 °C until they could be analyzed for infectious virus or they were stored at −80 °C until they could be analyzed for viral RNA.

RNA was extracted from each sample type using a QIAamp Minelute virus spin kit (Qiagen) executed on a Qiacube liquid handling device. RNA was kept on ice after extraction, and qRT-PCR reactions were set up on the same day as extraction. qRT-PCR reactions were set up using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (ThermoFisher Scientific). Taqman chemistry was used for the qRT-PCR assays using primer/probe sets from the Center for Disease Control and Prevention Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel designed at the US Center for Disease Control and Prevention for the detection of influenza B and for the detection and subtyping of influenza A. Reactions were run on an MX3005P qPCR system (Agilent Genomics) and consisted of a 30-min RT step at 50 °C, RT inactivation step, and 45 cycles of 95 °C/55 °C and fluorescence was read at the end of each extension step. Whole virion standards (Advanced Biotechnologies) that had been quantitated by electron microscopy were used to generate standard curves, and those standard curves were calibrated for copy number against plasmid DNA containing the targets of either the influenza A or influenza B qRT-PCR reaction.

Virus culture of the NP swabs and fine aerosol fractions on MDCK cells was used to identify samples with infectious virus. Culture was performed within 12 h of sample processing, and cell monolayers were observed on the fourth day postinoculation. Samples that did not exhibit cytopathic effect on the fourth day postinoculation were transferred to fresh cell monolayers and incubated for an additional 4 d. Monolayers that exhibited cytopathic effect on either the fourth or the eighth day postinoculation were considered infectious virus-positive. Samples with bacterial or fungal contaminants or not processed within 12 h were rejected. Only the results of valid samples processed within 12 h and without evidence of contaminants were included in reported data. Samples from the course-aerosol fraction were not cultured, as they were not expected to contain infectious virus given the collection conditions of that aerosol fraction.

Infectious virus in the NP swab and the concentrated fine-particle aerosol was quantified using an immunofluorescence assay as described previously with modification (1). Initial experiments were carried out in 96-well plates; however, we had greater success with this assay in 24-well plates. MDCK cells were incubated with the samples at room temperature for 1 h, and then the temperature was shifted to 37 °C to allow for virus entry to occur. After an hour at 37 °C, medium containing FBS was added to the culture and the cells were incubated for an additional 8 h. The addition of the serum serves two purposes. First it helps to maintain integrity and morphology of the cells, and it limits infection to a single round of replication by inactivating extracellular trypsin in the culture that is required for the cleavage of influenza's hemagglutinin in this system. In the absence of that cleavage event, virus particles are not infectious. Cells were fixed with ice-cold 80% acetone, and were stained for influenza A and B nucleoprotein using primary antibodies AA5h and sc-57885 (Abcam and Santa Cruz Biotechnoloy, respectively) followed by a goat anti-mouse secondary antibody conjugated with Alexa-Fluor488 (ThermoFisher Scientific). Positive cells were counted by fluorescence microscopy and reported as FFUs in the original sample.

EMIT Team Members

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1. Rudnick SN, McDevitt JJ, First MW, Spengler JD (2009) Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations. *Am J Infect Control* 37:813–819.

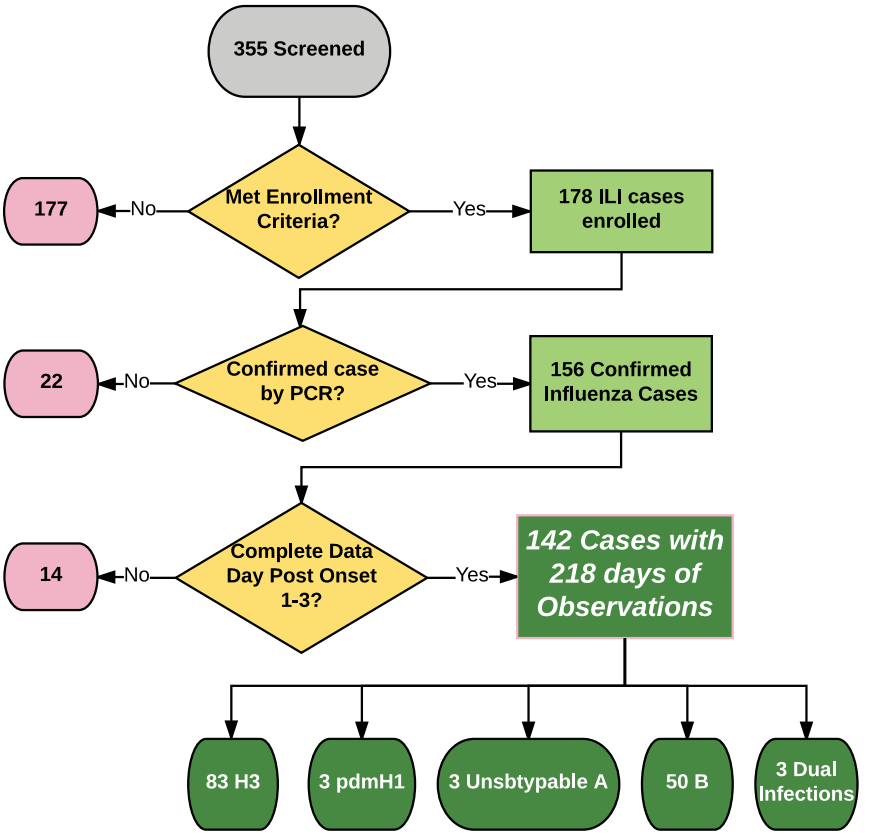

Fig. S1. Screening, enrollment, exclusions, and composition of final study population.

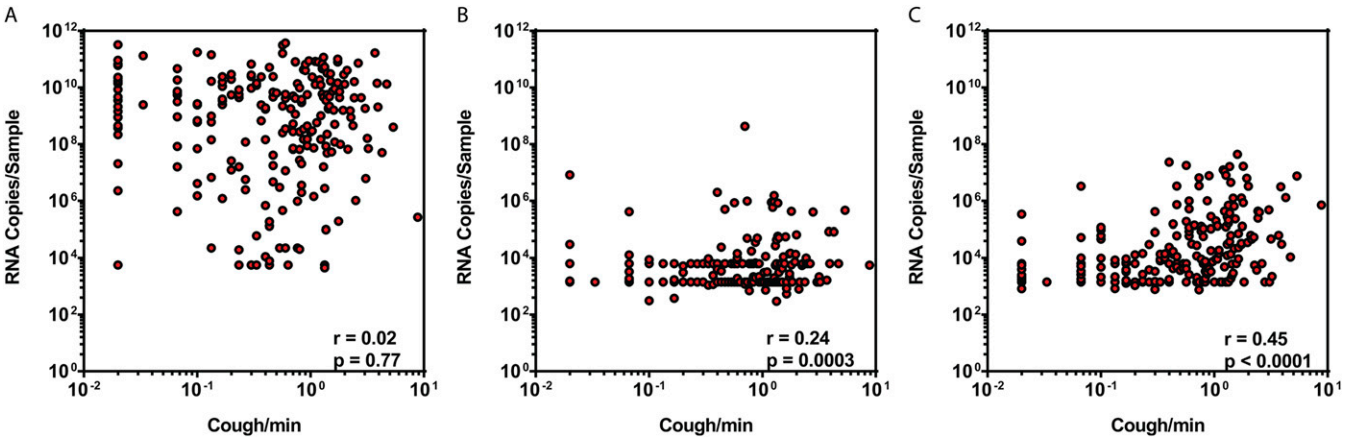

Fig. S2. Scatter plots and Spearman correlation coefficients of RNA copies plotted against cough per minute for NP (*A*), coarse (*B*), and fine (*C*) samples.

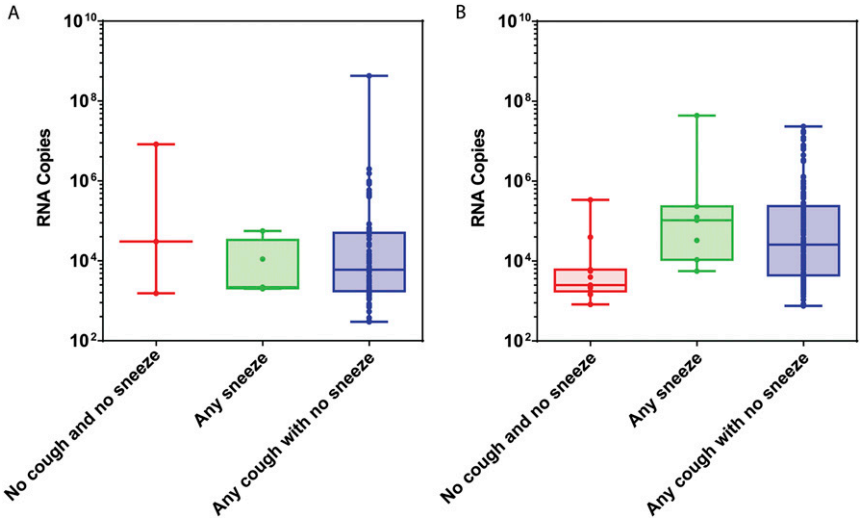

Fig. S3. The effect of sneeze: (*A*) RNA copies stratified by observed number of sneezes in coarse aerosols, and (*B*) in fine aerosols. Coarse aerosol = droplets >5 μm; and fine aerosol = droplets ≤5 μm in aerodynamic diameter.

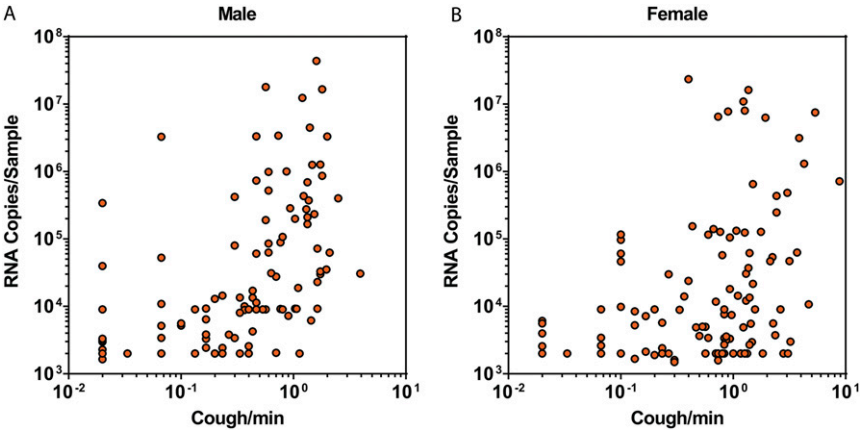

Fig. S4. Scatter plot showing male (*A*) and female (*B*) cough number vs. fine-aerosol RNA copies.

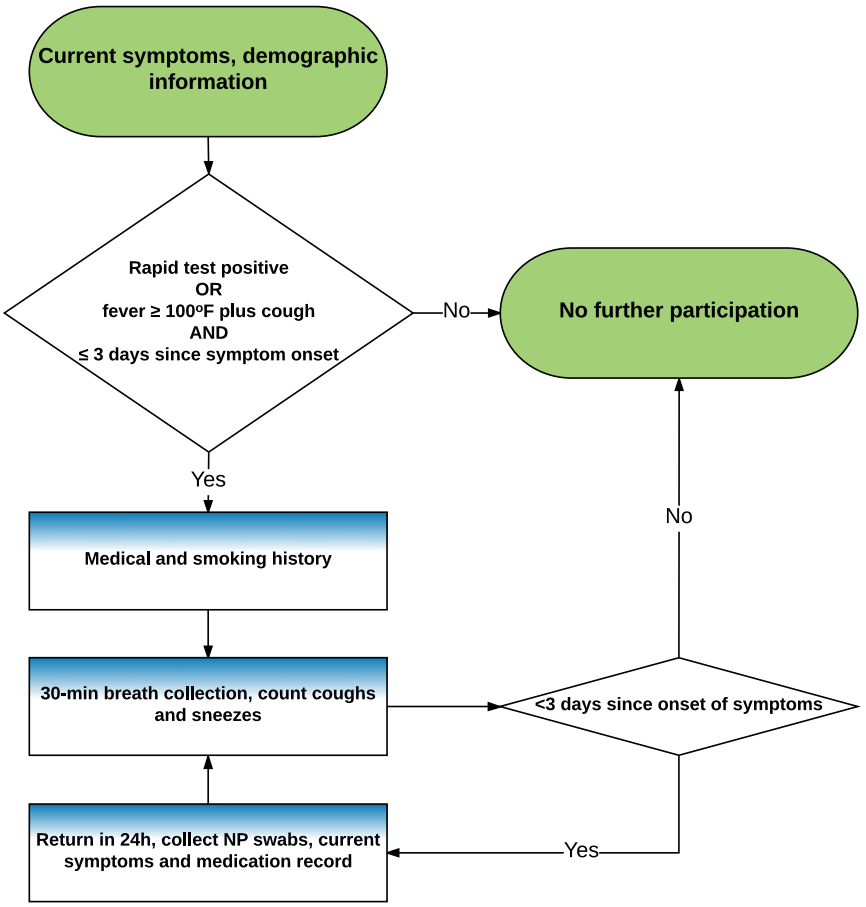

Fig. S5. Screening and sampling protocol.

Table S1. Summary of ILI case inclusion and exclusion for data analysis

Category	Subjects (*n*)	Breath collection visits (*n*)
Total ILI cases enrolled	178	278
Not confirmed by PCR*	22	32
Excluded visits on day 0[†,‡]	4	8
Excluded visits on day >3[‡]	7	10
Missing data for cough[‡]	2	7
Incomplete PCR data[‡]	1	3
Total excluded from analysis	36	60
Total included	142	218

ILI, influenza-like illness.

*All breath-collection visits for subjects who were not confirmed to have had influenza infection were excluded.

[†]Day 0 = day of symptom onset; day >3 = more than 3 d after symptom onset.

[‡]Many of the subjects with PCR confirmed infection who had a breath collection visit excluded for one or another reason had other visits with complete data that were included in the analysis. Thus, the total number of subjects and visits excluded do not match across rows.

Table S2. BMI of study population

Variable	Screened only (%)	Enrolled (%)	Complete data (%)
No. of participants	177	178	142
Underweight BMI <18.5	2 (1)	10 (6)	10 (7)
Normal BMI 18.5 to <25	119 (67)	113 (63)	91 (64)
Overweight BMI 25 to <30	38 (21)	40 (22)	29 (20)
Obese BMI ≥30	18 (10)	15 (8)	12 (8)

Table S3. BMI category and viral RNA shedding in fine aerosol

Parameter	Unadjusted	Adjusted
Underweight	0.25 (0.03–2.46)	0.59 (0.08–4.18)
Normal	Ref	Ref
Overweight	3.0 (0.70–12.9)	2.0 (0.53–7.14)
Obese	1.5 (0.17–13.5)	3.6 (0.53–24.4)

Effect estimates are shown as the ratio of BMI to Normal BMI group (95% CI for the effect estimate). All analyses are controlled for random effects of subject and sample within subject and for censoring by limit of detection using Tobit regression. Adjusted models were adjusted for influenza vaccination in the current and previous season, day of onset, number of coughs, and a sex by cough interaction. Ref, reference category.

Table S4. Effect of vaccination on unadjusted analysis of fine-aerosol viral RNA by type of influenza infection

Flu type	Total observed/(vaccinated)	Unadjusted (95% CI)	*P* value	Adjusted (95% CI)	*P* value
Flu A (current year vaccination)	91/(15)	4.6 (0.87–23.4)	0.07	5.8 (1.1–29.6)	0.03
Flu A (previous year vaccination)	91/(31)	2.9 (0.76–11.0)	0.12	3.0 (0.80–11.3)	0.10
Flu A (current and previous)	91/(11)	7.2 (1.1–45.7)	0.04	7.6 (1.2–47.2)	0.03
Flu B (current year vaccination)	51/(16)	1.2 (0.22–6.9)	0.81	0.94 (0.18–4.9)	0.94
Flu B (previous year vaccination)	51/(17)	1.4 (0.26–7.9)	0.67	1.3 (0.26–6.5)	0.75
Flu B (current and previous)	51/(11)	1.7 (0.24–11.2)	0.60	1.2 (0.19–7.7)	0.83

Effect estimates are the ratio of vaccinated to unvaccinated. Adjusted analyses account for age and sex and all analyses are controlled for random effects of subject and sample within subject and for censoring by limit of detection using Tobit regression.

Table S5. Limits of detection (LOD) and limits of quantification (LOQ) for qPCR reactions and calculated limits for the original samples taken

			Copies per sample			
	Copies per reaction		NP swab		Aerosol	
LOD and LOQ	Influenza A	Influenza B	Influenza A	Influenza B	Influenza A	Influenza B
LOD	20	20	200	200	500	500
LOQ	80	360	8,000	36,000	2,000	9,000

Limits were identified by making serial dilutions of a virus standard that has been calibrated against a plasmid standard for copy number per virus particle. The LOD represents the most dilute sample that gave a positive result in any replicate. The LOQ represents the most dilute sample that gave a positive result in all replicates.