

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

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Methods

The Seattle Flu Study is a multi-arm surveillance platform established in November 2018 to develop a community-based early detection system for influenza and other respiratory viruses.¹ Individuals were recruited into the study through online advertising and social media. Participants in the prospective community surveillance arm were eligible to participate if they were a resident within the greater Seattle metropolitan area (**Supplemental Figure 1**), self-reported new or worsening symptoms of acute respiratory illness (ARI) and were able to provide informed consent, or if a minor, had a legal guardian who could provide this. Eligible ARI symptoms included two of the following: feeling feverish, headache, sore throat or itchy/scratchy throat, nausea or vomiting, rhinorrhea, fatigue, myalgia, dyspnea, diarrhea, ear pain or ear discharge, rash, or a new or worsening acute cough alone. After signing an electronic consent form, all participants completed an electronic questionnaire to ascertain demographic, clinical, and behavioral characteristics using REDCap electronic data capture hosted at University of Washington.²

Online community enrollment occurred as part of two studies. As part of the “Swab-and-Send” study, individuals meeting eligibility criteria enrolled online through the study website (seattleflu.org) within seven days of symptom onset.³ As part of a household study of respiratory illness in families with children, participants enrolled online in longitudinal household studies before illness onset, and underwent weekly symptom monitoring as prompted by text message or e-mail reminders.⁴ When they met criteria for ARI, they self-collected (or had a parent or guardian collect) a mid-nasal swab based on instructions provided in the kit that was mailed to the participant’s home (**Supplemental Figure 2**). For participants enrolled in “Swab-and-Send” in the Seattle area, kits were mailed using FedEx Same Day City 2-hour delivery services, and returned via 2-day mail (**Supplemental Table 1**). For participants in the household study, the kits were delivered to the household at enrollment, and swabs were collected at the time of illness.

Samples were shipped according to standard requirements for shipping Category B biological substances.

This study was approved by the University of Washington Human Subjects Institutional Review Board (UW IRB).

Laboratory Methods

Prospective community surveillance samples were transported to the University of Washington laboratory in Universal Viral Transport Medium (Becton Dickinson, Franklin, NJ) at room temperature, and aliquoted and stored at 4°C prior to testing.

Laboratory testing was performed at the Brotman Baty Institute for Precision Medicine and the Northwest Genomics Center. Total nucleic acids were extracted (MagnaPure, Roche) and tested for the presence of 27 respiratory pathogens by TaqMan RT-PCR on the OpenArray platform (ThermoFisher) as well as SARS-CoV-2 (**Supplemental Table 2**).⁵ Because the CDC assay is low throughput, requiring 4 separate RT-PCR assays in individual wells of a 96-well plate, we developed a research assay using one SARS-CoV-2 probe set multiplexed with an RNase P, a human cellular marker, with a different fluor. Using this research assay 190 samples were tested in duplicate, in a single 384-well plate with no-template and positive template controls. The RNase P Ct values were used as an extraction and sample collection control. SARS-CoV-2 detection was performed using real-time RT-PCR with a SARS-CoV-2 probe set targeting Orf1b with FAM fluor (Life Technologies 4332079 assay # APGZJKF or the Centers for Disease Control N1 probe set from Integrated DNA Technologies) and RNaseP probe set with VIC fluor (Life Technologies A30064) on a QuantStudio 6 or Viiia7 instrument (Applied Biosystems). We added a Laboratory Developed Test (LDT) on March 19 which included another assay targeting the SARS-CoV-2 S gene ((Life Technologies 4332079 assay # APXGVC4), also performed in duplicate and multiplexed with RNase P-VIC. Three or four replicates for RNase P and SARS-CoV-2 must have

cycle threshold (Ct) < 39 for a sample to be considered positive in the LDT or both replicates must be positive for the research assay.

For assay validation, we first evaluated cross-reactivity among 270 samples collected prior to December 2019 that tested positive for other common respiratory viruses (human coronaviruses HCoV HKU1, NL63, OC43, 2293; influenza A/B, RSV A/B, rhinovirus, enterovirus, parainfluenza viruses 1-4, human metapneumovirus, and adenovirus). No samples tested positive for SARS-CoV-2 using this assay. Additionally, to account for the shipping time between sample collection and testing, we evaluated the stability of detection of SARS-CoV-2 from mid-nasal swabs collected according to the above methods. Samples were incubated for stability studies for a total of 9 days and analyzed at days 3, 5, 7, and 9 at 4°C, 20°C (room temperature in Seattle, WA, USA), and 28°C (average high temperature in Seattle in the summer) (**Supplemental Figure 3**). Assay performance was concordant with a maximum coefficient of variation of 3% for SARS-CoV-2 Ct values over all time and temperatures evaluated.

Data Analysis

Race and ethnicity categories were not mutually exclusive, as participants could select all options that were true for them. Smoking status was defined as the use of cigarettes, cigars, and pipes or electronic cigarettes/vapor pens. Underlying respiratory conditions were defined as chronic obstructive pulmonary disease (COPD), asthma, or chronic bronchitis. Other underlying chronic conditions included diabetes and cardiac disease. Chronic conditions were ascertained via self-report. Interference with daily activities was self-reported on a 5-point scale, with 1 representing no interference and 5 representing significant interference. A medical visit was defined as having sought care from a healthcare provider at a clinic, urgent care center, hospital, or emergency department prior to study enrollment for the participant's current respiratory illness. We generated descriptive statistics for all variables. Proportion of missing data are reported and percentages

are computed based on non-missing results. Statistical comparisons between groups were determined using Fisher's exact tests, and Wilcoxon-rank-sum tests, as appropriate. Linear regression was used to test for time trends. Two-tailed tests were used for all comparisons and statistical significance was defined as $p < 0.05$. Home residential addresses were converted to GPS coordinates, which were then assigned to Public Use Microdata Areas (PUMAs) to be mapped at an aggregated level.

Results

Overall demographics

From January 1 through March 9, 2020, 3,524 participants provided specimens via online enrollment. Of these, 2,353 (66.8%) completed all study procedures and had their samples tested. Those who completed all procedures had higher education, higher income and were more likely to have received influenza vaccine. There was no significant difference in time since symptoms started, having insurance, impact on regular activities, recent travel, household size, presence of chronic illness or seeking medical care prior to enrollment for current illness.

The 2,353 community surveillance participants included 2,089 (88.8%) from the "Swab-and-Send" study and 264 (11.2%) from the household studies. Of the 2,089 "Swab-and-Send" study participants, 1,316 received their swab kits via two-hour delivery (**Supplemental Table 1**). The mean delivery time overall was 2.5 hours [SD: 1.2], and 87% of the two-hour delivery kits arrived on-time. The median days from swab collection to receipt at the laboratory was 2.8 days [SD: 1.8].

Subjects were enrolled throughout the entire greater Seattle metropolitan area, including much of King County with some contribution by Snohomish County, located directly north of King County (**Supplemental Figure 1**). Compared to King County census data, the geographic distribution of

sampling overrepresented the northern parts of the region.

Samples were collected from 2353 participants (**Supplemental Table 4**). The mean age of those enrolled was 35.6 years [SD: 15.7]; 278 (11.9%) were children. Overall, both the youngest and oldest age strata were underrepresented as compared to King County census data (data not shown). The median household size of participants was 2 individuals [IQR: 2,4], and the majority were white (n=1916; 81.4%), and had private health insurance (n=2096; 89.1%). A total of 428 (18.2%) reported an underlying chronic respiratory condition; 17 (1.1%) were pregnant. Overall detection rates of respiratory viruses included 442 (18.8%) with influenza, 245 (10.4%) with rhinovirus, and 242 (10.3%) with human coronavirus. A total of 1027 (43.6%) were sick for two days or less at the time of sample collection, and 586 (24.9%) reported seeing a provider in the previous week for their ARI.

SARS-CoV-2 was detected in samples from 25 (1.1%) participants, including two subjects under 18 years of age (**Supplemental Table 4**). The first case of SARS-CoV-2 was detected on February 24, 2020. Mean semi-quantitative viral load as measured by cycle threshold of SARS-CoV-2 cases was 26.7 [SD: 4.6]. Coinfection with rhinovirus was present in four cases. None of the COVID-19 cases had influenza, respiratory syncytial virus, parainfluenza 1-4, other human coronaviruses, human metapneumovirus, adenovirus, or enterovirus co-detected.

The mean age of the 25 individuals with SARS-CoV-2 was 38.1 years (SD:15.1), 13 (52.0%) were female, and 5 (20.0%) reported an underlying chronic respiratory condition. Nine (36.0%) had fewer than two days of symptoms at the time of sample collection. Only 7/25 (28.0%) of individuals with COVID-19 reported seeking clinical care for their illness in the previous week. The most commonly reported symptoms included fatigue (21/25; 84.0%), myalgia (19/25; 76.0%), subjective fever (17/25, 68.0%), cough (17/25, 68.0%) and chills (17/25, 68.0%) (**Supplemental**

Figure 4). In those who did not have SARS-CoV2 detected, the most common symptoms were fatigue (1753/2322; 75.5%), rhinorrhea (1595/2322; 68.7%), cough (1530/2322; 65.9%), and sore throat (1522/2322; 65.5%).

Limitations

A limitation of this study is incomplete sampling of our community. We utilized convenience sampling for enrollment, with likely under-sampling among those with language barriers or lack of internet knowledge or availability. We targeted advertisements where we hoped to recruit under-represented minorities, but despite this, we likely undersampled non-English speaking, illiterate, or otherwise disadvantaged and/or individuals experiencing homelessness. In particular, online recruitment may have led to under-recruitment of the very young and elderly as compared to the county's population. However, widespread asymptomatic carriage of SARS-CoV-2 in children does not appear to be common overall, at least early in this pandemic.⁶ An additional limitation is the use of a collection method that has not been previously utilized for detection of SARS-CoV-2. Participants who enrolled in our study were provided instructions on a card to obtain a self or parent or guardian-collected mid-nasal swab, which was subsequently packaged and shipped to the laboratory at ambient temperature prior to testing. This collection method may have resulted in decreased sensitivity for detection of SARS-CoV-2 as compared to a gold standard of provider-collected nasopharyngeal swab both due to the unsupervised self-collection strategy and the potential degradation during shipping. However, we show data that supports stability of viral detection over time and across various temperatures, and concordance to nasopharyngeal swabs (**Supplemental Table 3**). Additionally, this novel collection method enabled collection of samples early in the onset of illness, as well as in participants who did not seek clinical care for their respiratory illness and would not have been identified through traditional clinical surveillance platforms.

Conflicts of Interest

HYC reports consulting with GlaxoSmithKline and Merck, and received research funding from Sanofi Pasteur, Cepheid, and Ellume. JAE reports consulting with Sanofi Pasteur and Meissa Vaccines, and research support by AstraZeneca, Chimerix, GlaxoSmithKline, Merck, and Novavax. Michael Boeckh reports consulting and research support by Gilead Sciences, Janssen, Ansun Biopharma, GlaxoSmithKline, Vir Biotechnology; and consulting with Pulmocyt, ADMA Biologics, and Moderna. MLJ has received research funding from Sanofi Pasteur, unrelated to the present work. JS reports consulting with Guardant Health, Maze Therapeutics, Camp4 Therapeutics, Nanostring, Phase Genomics, Adaptive Biotechnologies, and Stratos Genomics, and research collaboration with Illumina, all unrelated to the present work. MT has received research funding from Roche Molecular Diagnostics and consulting work for Click Diagnostics and Alere Inc.

DJM, TB, EB, DAN, JPH, CRW, JLog, TRS, KL, AA, PDH, MI, JLee, KF, BL, RB, LMS, CML, JH, CG, MF, AEK, and MJR report no conflicts of interest.

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Tables

Supplemental Table 1. Delivery logistics for the subset of samples delivered at time of illness (n=2,089) from the “Swab-and-Send” arm of the Seattle Flu Study, January 1 – March 9, 2020.

Delivery Counts		Delivery Averages	
Total deliveries to participants (all couriers) ^a	n=2089	Average delivery time (hours) to participants [SD]; range ^c	2.5 [1.2] (0.4 - 8.2)
Total FedEx Same Day City deliveries to participants ^b	n=1316	Average on-time percentage for deliveries to participants ^d	87%
		Average time (days) from nasal swab collection to receipt at the study laboratory [SD]; range ^e	2.8 ± 1.8 (0.0 – 14.0)

^a Various couriers were used to deliver self-swab kits depending on geographical location determined by zip code.

^b FedEx Same Day City was used to rapidly deliver self-swab kits within the city of Seattle.

^c Average delivery time for Seattle participants after adjusting for redeliveries.

^d 87% of FedEx Same Day City deliveries met the target delivery time of 2 hours.

^e Time between the completion of the nasal swab collection survey and receipt at the study laboratory. Proof of delivery (POD) reporting was not available for return shipments. Kits that were returned more than 14 days after enrollment were excluded under the assumption that these kits were used for a subsequent illness episode.

Supplemental Table 2. Pathogens for which all Seattle Flu Study respiratory specimens are tested using a TaqMan RT-PCR.

Viruses	Bacteria
Influenza A—H3N2	<i>Streptococcus pneumoniae</i>
Influenza A—H1N1	<i>Mycoplasma pneumoniae</i>
Influenza A—Pan	<i>Chlamydia pneumoniae</i>
Influenza B	<i>Bordetella pertussis</i> ^a
Influenza C	
Respiratory syncytial viruses A and B	
Parainfluenza viruses 1-4	
Coronavirus 229E, NL63, OC43, and HKU1	
Adenovirus	
Rhinovirus	
Measles	
Mumps	
Human metapneumovirus	
Human parechovirus	
Enterovirus ^b	
Enterovirus D68	
Human Bocavirus	

^a Includes *Bordetella bronchiseptica* and *Bordetella parapertussis*

^b All enterovirus species A, B, C, D, and G, including: all Coxsackie serotypes under species A, B, C; all Echovirus serotypes; all Poliovirus serotypes (1-3).

Supplemental Table 3. Summary of virological outcomes for clinical specimens in patients positive for SARS-CoV-2 in non-paired samples from nasopharyngeal and self-collected midnasal swabs.

Sample Type	Analysis	Orf1b gene	S Gene	RNase P internal control
Midnasal Swab	Number positive (%)	32 (100%)	32 (100%)	32 (100%)
	Mean Ct (SD)	28.4 (4.6)	28.6 (4.3)	24.2 (2.9)
Nasopharyngeal swab	Number positive (%)	10 (100%)	10 (100%)	10 (100%)
	Mean Ct	24.5 (8.1)	22.0 (1.6)	22.0 (1.6)

Supplemental Table 4. Demographic and clinical characteristics of study participants enrolled between January 1 - March 9, 2020, stratified by SARS-CoV-2 detection status.

Characteristic	SARS-CoV-2 Detection Status		
	Total <i>n</i> =2353	Positive <i>n</i> =25	Negative <i>n</i> =2328
Age, years (Mean, [SD])	35.6 [15.7]	38.1 [15.1]	35.9 [15.7]
Individuals <18 years [%]	278 [11.9]	2 [8.0]	276 [11.8]
Household size (Median [IQR])	2 [2,4]	2 [2,4]	2 [2,4]
Female [%]	1516 [64.5]	13 [52.0]	1503 [64.6]
Pregnant	17 [1.1]	0 [0.0]	17 [1.1]
<u>Race</u>			
Asian	371 [15.8]	3 [12.0]	368 [15.8]
Black	44 [1.9]	0 [0.0]	44 [1.9]
White	1916 [81.4]	23 [92.0]	1893 [81.3]
Other	114 [4.8]	0 [0.0]	114 [4.8]
Unknown	46 [2.0]	0 [0.0]	46 [2.0]
Hispanic/Latino	121 [5.2]	1 [4.0]	120 [5.2]
<u>Insurance Type</u>			
Government	195 [8.3]	5 [20.0]	190 [8.2]
Private	2096 [89.1]	20 [80.0]	2076 [89.2]
Smoker	108 [4.6]	2 [8.0]	106 [4.5]
<u>Underlying comorbidities</u>			
Respiratory ^a	428 [18.2]	5 [20.0]	423 [18.2]

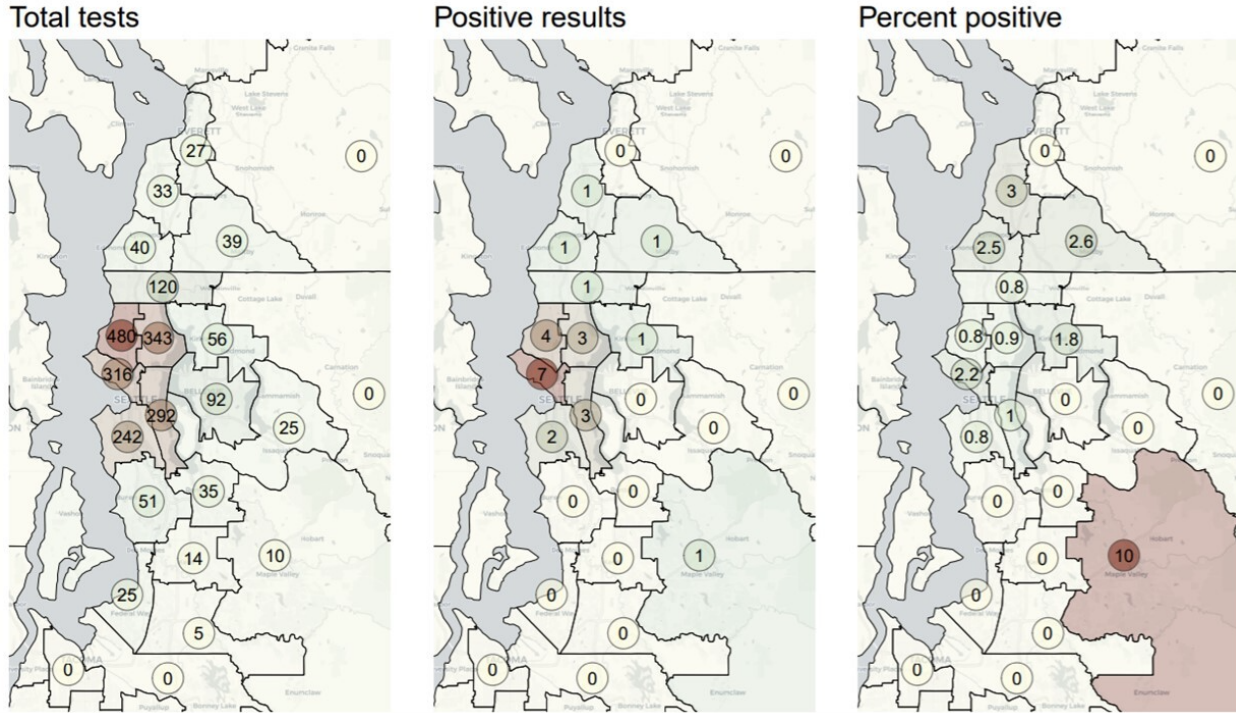
Other	55 [2.3]	0 [0.0]	55 [2.4]
<u>Other viruses detected</u>			
Influenza A/B	442 [18.8]	0 [0.0]	442 [19.0]
Respiratory syncytial virus	80 [3.4]	0 [0.0]	80 [3.4]
Rhinovirus	245 [10.4]	4 [16.0]	241 [10.3]
Human metapneumovirus	60 [2.5]	0 [0.0]	60 [2.6]
Human coronaviruses	242 [10.3]	0 [0.0]	242 [10.4]
Parainfluenza 1-4	21 [0.9]	0 [0.0]	21 [0.9]
Adenovirus	30 [1.3]	0 [0.0]	30 [1.3]
Enterovirus	17 [0.7]	0 [0.0]	17 [0.7]
<u>Days sick at sample collection</u>			
<2 days	1027 [43.6]	9 [36.0]	1018 [43.7]
3-4 days	821 [34.8]	8 [32.0]	813 [34.9]
>4 days	505 [21.5]	8 [32.0]	497 [21.3]
Interference with daily activities score ^b	3.4 [1.3]	3.7 [1.0]	3.4 [1.3]
(Mean [SD])			
Provider visit in prior week	586 [24.9]	7 [28.0]	579 [24.9]

^a Respiratory conditions include chronic obstructive pulmonary disease, chronic bronchitis, and/or asthma

^b Interference with daily activities was self-reported on a 5-point scale with 1 representing no interference and 5 representing significant interference.

Figures

Supplemental Figure 1. Total tests, positive results, and percent of tests returning a positive result for US Census Public Use Microdata Areas (PUMA) in King and Snohomish Counties.



Supplemental Figure 2. “Quick Start” instructions for self swab collection for “Swab-and-Send” Study.



Quick Start Guide

Thank you for helping us learn more about the flu!

We're sorry you're not feeling well. Please follow the steps outlined in this guide for taking a swab and mailing it back to us. We hope you get well soon!

If you have any questions, you can contact the study team at any time at: seattleflu@uw.edu or 206-221-4588.

STEP 1:

Fill out your survey

- 1 Search your inbox and spam folder for "Seattle Flu Study." **Click on the survey link.**
- 2 Fill out the survey on your web browser.

Step 2: Collect your Nasal Swab

- 1 Remove the swab from its packaging



- 2 Loosen and remove the red cap from the tube. *Careful!* - the tube contains liquid.



- 3 Insert swab halfway up the nose (about 1 inch).

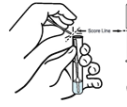
- 4 Press swab against the side and rotate swab 5 times.



- 5 Place the swab into the solution in the provided tube.



- 6 Break the swab handle at the score line (break line) by bending back and forth.



- 7 Screw red cap on tightly.



STEP 3:

Ship your nasal swab

- 1 Write your name & the date on the collection tube.
- 2 Place your tube into the specimen bag & seal it tightly.
- 3 Place the specimen bag back into the box provided.
- 4 Place the box into the prepaid return bag.
- 5 Seal the bag by removing the adhesive strip.
- 6 **Mail it back ASAP using:**
 - a. Your own mailbox (if it fits).
 - b. A USPS Blue Box or Post Office.
 - c. A scheduled Package Pickup on usps.com.

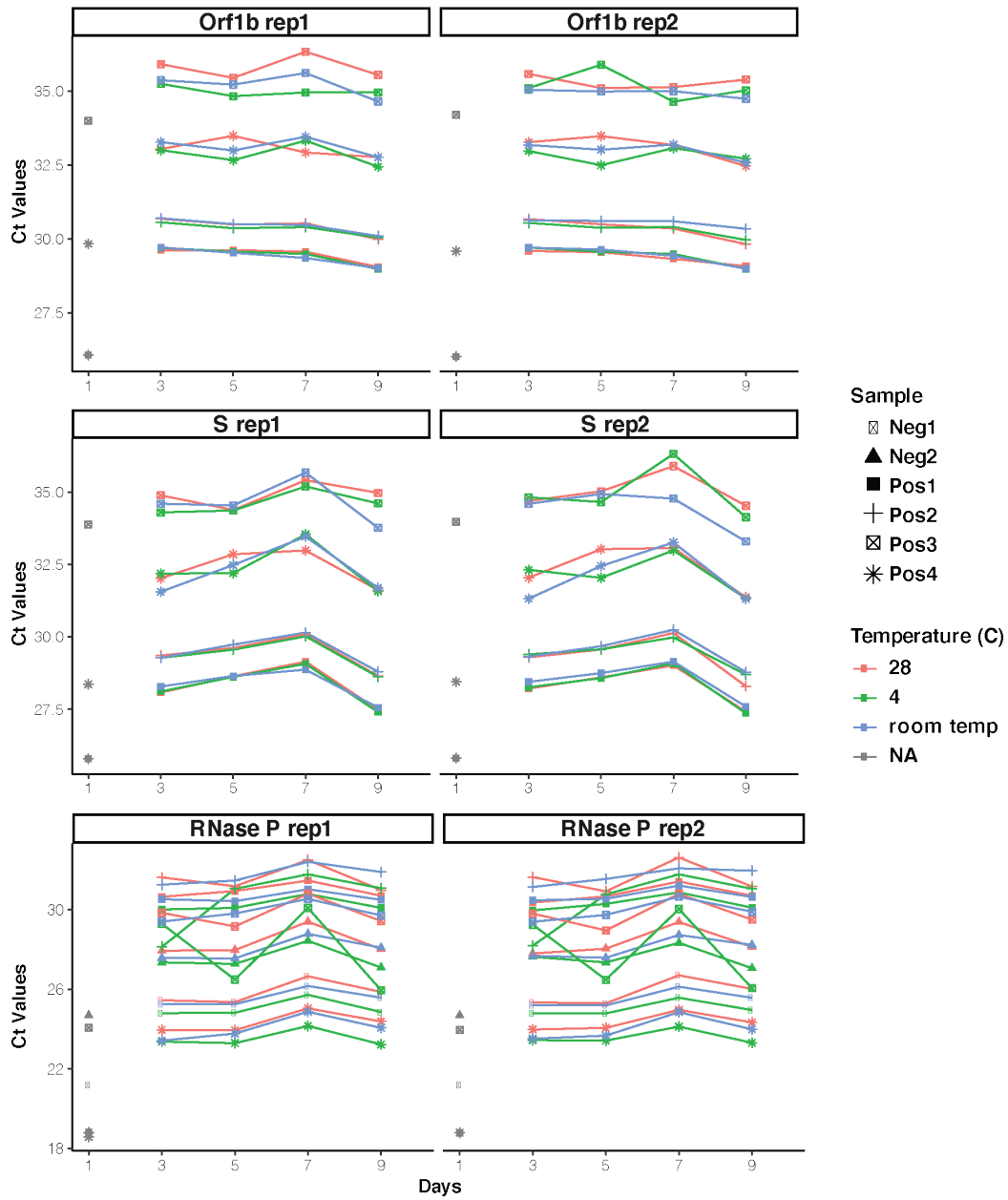
STEP 4:

Complete a follow-up survey online

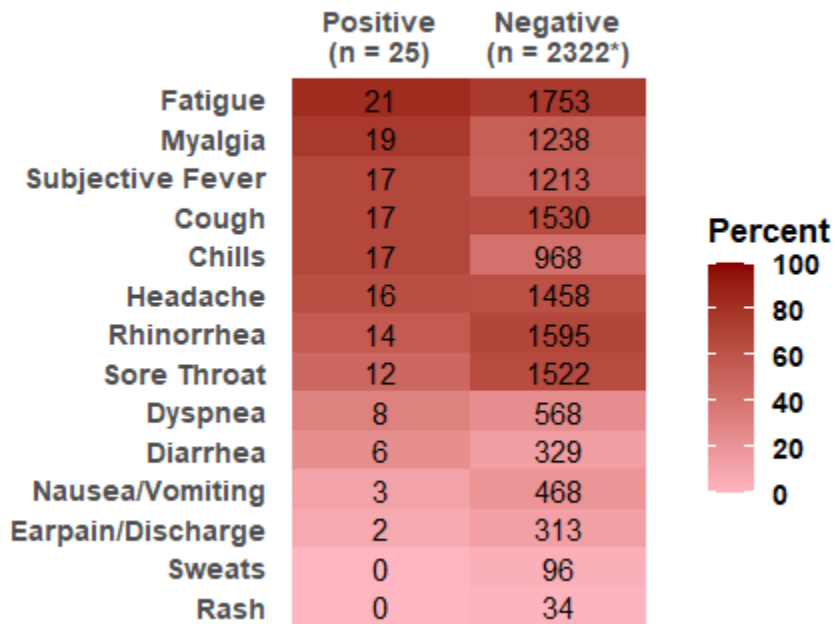
Be on the lookout for a follow-up survey, delivered via email in about 7 days.



Supplemental Figure 3. Stability of SARS-CoV-2 in universal transport media at multiple temperatures over time. Four SARS-CoV-2 positive and 2 SARS-CoV-2 negative participant samples (undiluted Ct value on day 1) were diluted 4-16 fold into universal transport medium and incubated at 4°C, room temperature or 28°C. 200 ul was extracted on the indicated days and SARS-CoV-2 Orf1b and S and human RNase P were detected by qRT-PCR in duplicate. Ct values are reported.



Supplemental Figure 4. Symptom heat map of enrolled participants stratified by SARS-CoV-2 detection status, January 1 - March 9, 2020.



*Symptoms missing in 6 of 2328 negative samples.