Supplemental Online Content

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Association of mRNA COVID-19 Vaccination with Clinical and Virologic Features of COVID-19 among US Essential and Frontline Workers

Supplementary Online Content

eMethods

Study Recruitment

The study sites included: University of Arizona in Tucson, Arizona, Baylor Scott and White Health in Temple, Texas; Kaiser Permanente Northwest in Portland, Oregon; the University of Miami in Miami, Florida; St. Luke's Hospital in Duluth, Minnesota; and the University of Utah in Salt Lake City. Sites utilized a stratified recruitment approach by sex, age group (18 – 39 years old and 40+ years old), and occupation (primary healthcare personnel (HCP) nurses and other allied healthcare personnel, first responders, essential and frontline workers). Each site aimed to recruit at a minimum of 20 participants per sex, age, and occupation category. Specific study site recruitment listed below.

Baylor

Participant catchment area included: Bell County and cities of Temple, Belton, and Killeen, Texas. HCP and first responders were recruited from past participants of other research studies and employees within the Baylor health systems. Essential and frontline workers were recruited from local worker groups association that had existing university partners.

Kaiser

Participant recruitment catchment area was from the Northwest Oregon area including from Eugene, Oregon to Longview, Washington. HPC and first responders were recruited from previous research participants and employees within the Kaiser medical coverage area. Essential and frontline workers were recruited from those with Kaiser medical coverage.

St. Luke's

The catchment area for participant recruitment was limited to within 100 miles of Duluth, including northwest Wisconsin. HCP were recruited from employees within the St. Luke's health system and first responders were recruited from first responders in the surrounding area. Essential and frontline workers recruited from various local worker groups.

University of Arizona

Participants were recruited with a community-based approach from Arizona residents, which included from ongoing COVID-19 testing activities (e.g. university-driven antibody and saliva testing initiatives or state-led serology surveillance), community-based COVID-19 cohorts that do not overlap in study scope, and self-referrals.

University of Utah

Participant recruitment catchment area included 60 miles within Salt Lake City, Utah. HCP were recruited from employees within the University of Utah health system and first responders were recruited from the first responders in the surrounding areas. Essential and frontline workers were recruited from local worker groups associated with existing university partners.

Laboratory: Qualitative and Quantitative RT-PCR and Genetic Sequencing Methods

Laboratory: Qualitative Real-time RT-PCR

Infection was diagnosed by RT-PCR positive from either nasal or saliva specimens. RNA extraction was performed using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher Flex system. RT-PCR was performed using the TaqPath™ COVID-19 Combo Kit on the QuantStudio 7 Pro real time RT-PCR system. Positive specimens were defined as having at least two SARS-CoV-2 targets (ORF1ab, N gene, S gene) with a threshold cycle (Ct) value ≤37 per manufacturer's instructions.[1] Approximately 20% of specimens were randomly selected for retesting as part of routine quality control testing procedures.

Laboratory: Quantitative SARS-CoV-2 RT-PCR

Residual positive specimens were frozen at -80 degrees Celsius and shipped on dry ice to the Wisconsin State Laboratory of Hygiene (WSLH) for quantitative SARS-CoV-2 RT-PCR. Specimens were extracted using a QIAcube HT with QIAmp 96 Virus extraction kit (PN 57731) and tested using the CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay (https://www.fda.gov/media/139743/download) on the ABI 7500 Fast Dx. SARS-CoV-2 and RNase P targets from this assay were utilized. This assay has Emergency Use Authorization as a qualitative real-time RT-PCR test. To make this assay quantitative, a standard curve of synthetic SARS-CoV-2 RNA (PN 102024, Twist Bioscience) was included on every ABI 7500 run. Starting with $1x10^6$ copies/µl, a 6-point standard curve of 10-fold dilutions were included on each PCR run, with each dilution run in triplicate (18 wells total). After the first run demonstrated a Ct Standard Deviation <1, specimens were tested once (in one well). The average Ct values of each dilution of standard were plotted using linear regression, and the linear regression equation was used to convert Ct values of specimens into log copies/µL for each specimen. Specimens with Ct values outside the standard curve were reported as <10 copies/µL or >1,000,000 copies/µL.

For quality control, one negative and one quantified positive control (Cat. NATSARS COV2-ERC, Zeptometrix Corp.) were included for each extraction and RT-PCR run. For a run to pass, the negative control must be negative, and the positive control must be within the range of mean+/- 3 standard deviations of the average Ct value of the positive control. In addition, to pass quality control the R-squared of the standard curve must be >0.97; the R-squared was consistently ≥0.99. For each specimen to pass, the RNase P Ct needed to be <35, indicating adequate human specimen collection; all specimens passed this minimal indicator of specimen quality.

Specimens with qualitative PCR cycle threshold (Ct) ≥30 were assumed to have an undetectable viral RNA load, since 83.6% (676/809) of specimens with Ct ≥30 had undetectable viral RNA in an initial evaluation using quantitative PCR. Additional details for these methods have been published previously [2].

Laboratory: Genetic sequencing

SARS-CoV-2 whole-genome sequencing was conducted at the Marshfield Clinic Research Institute and CDC, in accordance with previously published protocols [3]. Genetic sequence of the SARS-CoV-2 target region was analyzed to determine if genetic substitutions may have impacted genome copy calculations in vaccinated infections. No systematic substitutions were seen in the conserved SC2 target region. Viruses not eligible (cycle threshold [Ct] value ≥30) for sequencing were assumed to be the origin SARS-CoV-2 lineages until the Delta variant accounted for >50% of sequenced viruses at each study site. Similarly, viruses not eligible for sequencing were assumed to be the Omicron variant once they accounted for ≥50% of viruses sequenced.

Laboratory: Plaque forming units titration methods

All eligible Delta viruses were included. All eligible origin SARS-CoV-2 viruses among vaccinated participants were included; however, due to resource constraints, only up to two origin SARS-CoV-2 viruses from unvaccinated participant infections occurring within two weeks of vaccine breakthrough infections were tested.

The quantity of infectious virus particles was made by determination of plaque-forming units (PFU) per milliliter if the RT-PCR cycle threshold (Ct) was <30, viral RNA load was >10 log10 RNA copies/mL, and virus lineage had been confirmed. All eligible Delta viruses were tested, irrespective of participant vaccination status. Due to resource constraints, testing for origin SARS-CoV-2 viruses included all vaccinated participants, but only a subset from unvaccinated participants. We selected origin SARS-CoV-2 viruses from unvaccinated participants with a collection date that was within two weeks of a vaccine breakthrough infection; if more than two unvaccinated participant specimens were eligible for inclusion, specimens with the lowest Ct values were selected for testing.

Plaque assays were performed in Vero E6 cells expressing the TMPRSS2 T2A and human ACE2 receptors (provided by Barney Graham, Vaccine Research Center, National Institutes of Health) on a Puromycin resistance plasmid. Cells were plated at approximately $7.5x10^5$ cells/well in 6-well plates and incubated at 37°C and 5% CO_2 in Dulbeccos Modified Eagle's Medium – high glucose (DMEM) (Sigma D6429-500ML) supplemented with 10% FCS, 100 µg mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL Amphotericin B (100X Antibiotic-Antimycotic Gibco 15240-062), and Puromycin at 10 µg/ml. The following day, cells were washed with 1X PBS and incubated with infection media. Infection media was prepared using DMEM supplemented with 2% FCS, 100 µg/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL Amphotericin B (100X Antibiotic-Antimycotic Gibco 15240-062). Samples were then diluted either at 1:5, 1:50, and 1:500, or 1:5, 1:100, and 1:2000 in 300 µL of infection media. The infection media was removed from the cells and 250 µL of the sample titration was added. Cell plates

were incubated at 37℃ with 5% CO_2 for 1 hour and gently rocked side-to-side every 10-15 minutes to prevent the monolayer from drying.

Towards the end of the 1-hour incubation, 10 mL of molten 2% SeaPlaque low-melt Agarose (Lonza 50100) with 10 mL of 37℃ 2X MEM were added per 6-well plate. After the incubation, the virus dilution inoculum was aspirated and 3 mL/well of the 1% agarose-1X MEM overlay was gently added. The overlay was allowed to solidify at room temperature (~15 minutes) and then plates were incubated at 37℃ and 5% CO2 for 70-72 hours.

After the 3-day incubation, the plates were placed at 4℃ for about 30 minutes to increase firmness of the overlay. The overlay was then gently removed and 1-2 mL of 10% neutral buffer formalin was added. The plates were incubated for about 30 minutes at room temperature and the 10% neutral buffer formalin was aspirated. Each plate was gently rinsed with water to remove excess formalin. 0.1% crystal violet in 20% ethanol was added to each well to fully cover the fixed monolayer and the plates were incubated at room temperature for 1-hour. Following the incubation, the 0.1% crystal violet was aspirated, and the plates were gently rinsed with water until excess crystal violet was removed. The plates were then left uncovered to fully dry and plaques were counted using a light box.

Viral titers were calculated as PFU/mL = number of plaques x (dilution factor) x 4 (to get from 250 µL to 1 mL).

Statistical methods

Analytic sample

Participants with laboratory documented SARS-CoV-2 infections prior to the start date were excluded. Prior infections were identified from self-report of reverse-transcriptase-polymerase-chain reaction (RT-PCR) testing results prior to enrollment, from RT-PCR testing during the study, or from detection of neutralizing antibodies to SARS-CoV-2 from sera collected at enrollment or quarterly.

Participants who received only dose-1 or had an index date 1-13 days after dose-2 or 1-6 days after dose-3 were excluded from the analytic sample due to indeterminate vaccination status. Because of the relatively small number of Ad26.COV2.S (Janssen vaccine [Johnson & Johnson]) vaccine recipients in our cohort and the difference between mRNA and Ad26.COV2.S dose recommendations, Ad26.COV2.S recipients were excluded.

Characteristics of COVID-19 were described among participants who completed at least one illness survey. Those without an illness survey were excluded. A post-illness follow-up survey was collected for those reporting any days sick in bed or missed work during their illness; those without a post-illness survey were excluded from analyses on these two outcomes.

Outcomes

Illness characteristics

Participants were asked about the following 19 symptoms if they remained symptomatic 1 week after onset and upon illness resolution: Fever, Chills or a sudden feeling of cold with shaking, Cough, Sore Throat, Runny nose or nasal congestion, Shortness of breath or difficulty breathing, Chest pain or chest tightness, Muscle aches or body aches, Joint pain, Abdominal or stomach pain, Nausea or queasy feeling that you may vomit, Vomiting, Diarrhea (at least 3 loose stools in a 24 hour period), Headache, Fatigue (unusual feeling of tiredness), Rash, Changes to skin of fingers or toes, including change in color blistering, Eye redness, and Change in smell or taste. Total days of symptoms was trimmed to the 95th percentile to remove the influence of any potential long COVID-19 cases. Days spent sick in bed and hours of missed work were trimmed to the 99th percentile to remove the influence of illogical outliers (i.e., 100 days in bed for an infection with two days of symptoms).

Imputation of quantitative viral RNA load

As an alternative to using half of the lower threshold, we used the linear relationship between qualitative RT-PCT Ct values and log 10 viral RNA load to predict unquantifiable viral RNA load. This adds variability to the unquantifiable samples the half lower limit does not. It also allows for values close to zero which give the estimation curves for viral load over time the proper lower limit of zero rather than 5. The linear model log 10 viral RNA load= $\alpha + \beta$*(ct value) was built using all samples with a quantifiable viral RNA load. The model estimated intercept and

beta were then used to predict missing viral RNA loads using the qualitative RT-PCR Ct value. The final prediction equation was log 10 viral RNA load= 9.032 + -0.271*(ct value).

Viral RNA load time course

Viral RNA load by days from index date was estimated using a Bayesian hierarchical model. Participants were limited to those with at least 3 RT-PCR positive samples and at least one sample being quantified. The relationship between viral RNA load and days from index date was modeled with a three-knot natural spline, which allows the slope up and down from peak to curve. Separate models were built to look at viral time course by virus variant and symptomology. Interactions between the exposure and time from onset were checked but not significant. There were not sufficient participants in each group to look at time course by vaccination status. Time to peak and time to <1 (log10 copies/μL) were estimated using daily means of poster predicted values. Time to peak was the day of the highest predicted mean. Time to <1 (log10 copies/μL) was the first day the predicted mean was below 1 minus the predicted peak.

Sensitivity analysis

The time course analysis required at least one of the samples to be quantified. As a sensitivity analysis, the same model was run on the subset of participants with at least two samples quantified. The time to peak and time to undetectable viral RNA load did not change from the main analysis. The relationship between the vaccine strains also did not change with viral RNA load for origin SARS-CoV-2 and Delta not being different from each other but both higher than Omicron. Including participants with one quantified sample and two below threshold did not introduce appreciable bias to the analysis.

Supplemental Results

The characteristics of SARS-CoV-2 infected participants by vaccination status were examined in eTable1. The median age ranged from 40.3 – 42.0 years old. Most study participants were non-Hispanic White across all vaccination categories/ Those who were non-Hispanic were most likely to have their third dose while hose where were Hispanic of any race were more likely to have their second dose ,150 days or be unvaccinated. Among occupation groups, first responders were the most likely to be unvaccinated while Primary HCP were the least likely. Chronic health conditions were consistent across vaccination status but participants taking daily medications were more likely to be vaccinated. Participants received BNT162b2 (Pfizer-BioNTech) COVID-19 vaccine product most frequently among those vaccinated with at least 2-doses.

The socio-demographic and health characteristics were examined for all SARS-CoV-2 infected participants by virus lineage in eTable 2. Across all lineages, most were female, non-Hispanic white, did not report any chronic conditions, did not report any daily medications, and self-rated their health as excellent or very good. First responders had the highest number of origin infections. For Delta infections, the highest number of infections occurred equally in nurses and other allied healthcare works and first responder. Similarly, for Omicron infections, most infections occurred for nurses and other allied healthcare workers and essential/frontline workers.

When examining the illness characteristics for all SARS-CoV-2 infected participants, number of hours of missed work and receipt of medical care during illness differed by study site location (eTable 3). Females compared to males spent more days sick in bed on average. Compared to other occupations, first responders missed the most hours of work due to illness; nurses and other allied healthcare personnel spent the most days sick in bed and sought medical care for their illness most often. Participants with lower versus higher self-rated health (good/fair/poor versus very good/excellent) had longer illnesses, spent more days sick in bed, and sought medical less often for their illnesses; similar differences were noted for participants with one or more chronic medical conditions and participants who took 2 or more daily medications.

Viral RNA load was higher for those aged ≥50 years, male, other race and ethnicity, first responders, had ≥1 chronic condition, and took ≥2 daily medications. PFU count was lower among participants who were male, aged ≥50 years, had at least one chronic condition, or took 2 or more daily medications (eTable 4). Mean PFU count for symptomatic Delta infections was higher compared to asymptomatic infections (mean difference, 2.0 [(95% CI, 0.5 – 3.4]) and was higher for symptoms that lasted 14 days or more (mean difference, 1.1 [95% CI, 0.1 – 2.1]).

eTables

eTable 1. Characteristics of SARS-CoV-2 Infected Study Participants by Vaccination Status.

	Unvaccinated		Vaccinated 2-dose 14-149 days		Vaccinated 2-dose ≥ 150 days		Vaccinated 3-dose 7-149 days	
	N	(Col%)	N	(Col%)	N	(Col%)	N	(Col%)
Total	352		72		374		401	
Demographics								
Age (Years), median (IQR)	41.0	(34.0 - 50.1)	40.3	(31.5-50.0)	41.2	(35.0-50.4)	42.0	(35.0 - 50.8)
Sex								
Female	180	(51.1)	42	(58.3)	215	(57.5)	277	(69.1)
Male	170	(48.3)	30	(41.7)	159	(42.5)	124	(30.9)
Race and Ethnicity								
Hispanic of Any Race	82	(23.3)	18	(25.0)	75	(20.1)	57	(14.2)
Non-Hispanic Asian	6	(1.7)	0	(0.0)	6	(1.6)	10	(2.5)
Non-Hispanic Black	8	(2.3)	2	(2.8)	11	(2.9)	14	(3.5)
Non-Hispanic White	234	(66.5)	51	(70.8)	274	(73.3)	312	(77.8)
Other	7	(2.0)	1	(1.4)	3	(0.80)	6	(1.5)
Cohort location								
Phoenix, AZ	74	(21.0)	6	(8.3)	73	(19.5)	83	(20.7)
Tucson, AZ	123	(34.9)	11	(15.3)	92	(24.6)	92	(22.9)
Miami, FL	52	(14.8)	7	(9.7)	58	(15.5)	21	(5.2)
Duluth, MN	18	(5.1)	7	(9.7)	35	(9.4)	74	(18.5)
Portland, OR	18	(5.1)	8	(11.1)	17	(4.5)	47	(11.7)
Temple, TX	25	(7.1)	8	(11.1)	33	(8.8)	31	(7.7)
Salt Lake City, UT	42	(11.9)	25	(34.7)	66	(17.6)	53	(13.2)
Occupation [b]								
Primary HCP	17	(4.8)	4	(5.6)	51	(13.6)	115	(28.7)
Nurses and other allied HCP	87	(24.7)	26	(36.1)	108	(28.9)	127	(31.7)
First Responders	145	(41.2)	15	(20.8)	120	(32.1)	56	(14.0)
Essential and other frontline	103	(29.3)	27	(37.5)	95	(25.4)	103	(25.7)
Medical History								
Chronic Condition [a]								
None	248	(70.5)	50	(69.4)	271	(72.5)	285	(71.1)
1 or more	91	(25.9)	22	(30.6)	100	(26.7)	116	(28.9)
Number of medications								
None	175	(49.7)	37	(51.4)	190	(50.8)	187	(46.6)
1	69	(19.6)	14	(19.4)	72	(19.3)	95	(23.7)
2 or more	90	(25.6)	21	(29.2)	106	(28.3)	115	(28.7)
Self-Rated Health								
Excellent/Very good	231	(65.6)	51	(70.8)	265	(70.9)	306	(76.3)
Good/Fair/Poor	121	(34.4)	21	(29.2)	109	(29.1)	95	(23.7)
Vaccine product [c]								

BNT162b2 (Pfizer-BioNTech)	-	-	46	(63.9)	234	(62.6)	272	(67.8)
mRNA-1273 (Moderna)	-	-	26	(36.1)	140	(37.4)	107	(26.7)
Mixed dosing	-	-	-	-	-	-	22	(5.5)
SARS-CoV-2 Lineage								
Origin strains	165	(46.9)	3	(4.2)	0	(0.0)	0	(0.0)
Delta variant	78	(22.2)	27	(37.5)	165	(44.1)	18	(4.5)
Omicron variant	109	(31.0)	42	(58.3)	209	(55.9)	383	(95.5)

Abbreviations: Column (Col.), Interquartile range (IQR), Healthcare Providers (HCP)

a. Chronic condition included asthma, chronic lung disease, cancer, diabetes, heart disease or condition, immunosuppression, kidney disease, liver disease, neurologic or neuromuscular disease, autoimmune disease.

b. Primary HCP included physicians, physician assistants, nurse practitioners, and dentists; allied HCP included nurses, therapists, technicians, medical assistants, orderlies, and all others providing clinical support in inpatient or outpatient settings; first responders included firefighters, law enforcement, corrections officers, and emergency medical technicians; and other essential and frontline workers included teachers and hospitality, delivery, and retail workers, and other occupations requiring routine close contact with the public or coworkers.

c. Mixed dosing refers to participant's dose-3 vaccine manufacturer that differs from dose-1 and dose-2.

eTable 2. Characteristics of Participants with SARS-CoV-2 Infections by Virus lineage

	Total Infections		Origin strain		Delta variant		Omicron variant	
	N	(Col%)	N	(Row%)	N	(Row%)	N	(Row%)
Total	1199		168	(14.0)	288	(24.0)	743	(62.0)
Demographics								
Age (Years), median (IQR)	41	(35-50.5)	41	(33.8-51.0)	41.3	(35.2-51.0)	41	(35.0-50.3)
Sex								
Female	714	(59.5)	90	(53.6)	167	(58.0)	457	(61.5)
Male	483	(40.3)	78	(46.4)	120	(41.7)	285	(38.4)
Race and Ethnicity								
Hispanic of Any Race	232	(19.3)	41	(24.4)	55	(19.1)	136	(18.3)
Non-Hispanic Asian	22	(1.8)	2	(1.2)	4	(1.4)	16	(2.2)
Non-Hispanic Black	35	(2.9)	5	(3.0)	4	(1.4)	26	(3.5)
Non-Hispanic White	871	(72.6)	111	(66.1)	214	(74.3)	546	(73.5)
Other	17	(1.4)	3	(1.8)	4	(1.4)	10	(1.3)
Cohort locations								
Phoenix, AZ	236	(19.7)	41	(24.4)	62	(21.5)	133	(17.9)
Tucson, AZ	318	(26.5)	68	(40.5)	61	(21.2)	189	(25.4)
Miami, FL	138	(11.5)	18	(10.7)	30	(10.4)	90	(12.1)
Duluth, MN	134	(11.2)	9	(5.4)	43	(14.9)	82	(11.0)
Portland, OR	90	(7.5)	4	(2.4)	23	(8.0)	63	(8.5)
Temple, TX	97	(8.1)	18	(10.7)	19	(6.6)	60	(8.1)
Salt Lake City, UT	186	(15.5)	10	(6.0)	50	(17.4)	126	(17.0)
Occupation [b]								
Primary HCP	187	(15.6)	11	(6.5)	44	(15.3)	132	(17.8)
Nurses and other allied HCP	348	(29.0)	46	(27.4)	88	(30.6)	214	(28.8)
First Responders	336	(28.0)	65	(38.7)	88	(30.6)	183	(24.6)
Essential and other frontline	328	(27.4)	46	(27.4)	68	(23.6)	214	(28.8)
Medical History								
Chronic Condition [a]								
None	854	(71.2)	114	(67.9)	200	(69.4)	540	(72.7)
1 or more	329	(27.4)	48	(28.6)	84	(29.2)	197	(26.5)
Number of medications								
None	589	(49.1)	66	(39.3)	134	(46.5)	389	(52.4)
1	250	(20.9)	41	(24.4)	53	(18.4)	156	(21.0)
2 or more	332	(27.7)	54	(32.1)	94	(32.6)	184	(24.8)
Self-Rated Health								
Excellent/Very good	853	(71.1)	115	(68.5)	195	(67.7)	543	(73.1)
Good/Fair/Poor	346	(28.9)	53	(31.5)	93	(32.3)	200	(26.9)
Vaccine product [c]								
BNT162b2 (Pfizer-BioNTech)	325	(27.1)	1	(0.6)	136	(47.2)	401	(54.0)
mRNA-1273 (Moderna)	136	(11.3)	2	(1.2)	59	(20.5)	208	(28.0)
Mixed dosing	40	(3.3)	0	(0.0)	15	(5.2)	25	(3.4)

Abbreviations: Column (Col.), Interquartile range (IQR), Healthcare Providers (HCP), HEROES (the Arizona Healthcare, Emergency Response, and Other Essential Workers Surveillance Study), RECOVER (Research on the Epidemiology of SARS-CoV-2 in Essential Response Personnel)

a. Chronic condition included asthma, chronic lung disease, cancer, diabetes, heart disease or condition, immunosuppression, kidney disease, liver disease, neurologic or neuromuscular disease, autoimmune disease.

b. Primary HCP included physicians, physician assistants, nurse practitioners, and dentists; allied HCP included nurses, therapists, technicians, medical assistants, orderlies, and all others providing clinical support in inpatient or outpatient settings; first responders included firefighters, law enforcement, corrections officers, and emergency medical technicians; and other essential and frontline workers included teachers and hospitality, delivery, and retail workers, as well as all other occupations that require routine close contact with the public, customers, or coworkers.

c. Mixed dosing refers to participant's dose-3 vaccine manufacturer that differs from dose-1 and dose-2.

eTable 3. Participant characteristics by illness characteristics among SARS-CoV-2 infected adults

	SARS-CoV-2 Cases		Symptom duration		Hours of missed work		Days sick in bed		Febrile[a]		Afebrile[b]		Received medical care[c]		Did not receive medical care	
	No.	(%)	Mean	(SD)	Mean	(SD)	Mean	(SD)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Total	1199		13.7	(8.9)	38.8	(32.9)	3.0	(3.4)	672		318		245		741	
Demographics																
Age (Years)																
18 - 49	878	(73.2)	13.5	(8.7)	39.4	(31.8)	2.9	(3.4)	490	(72.9)	239	(75.2)	176	(71.8)	551	(74.4)
≥50	321	(26.7)	14.0	(9.6)	37.0	(35.8)	3.1	(3.3)	182	(27.1)	79	(24.8)	69	(28.2)	190	(25.6)
Sex																
Female	714	(59.5)	14.3	(9.1)	38.4	(32.2)	3.4	(3.6)	409	(60.9)	193	(60.7)	162	(66.1)	436	(58.8)
Male	483	(40.3)	12.7	(8.6)	39.5	(34.0)	2.2	(2.8)	262	(39.0)	125	(39.3)	83	(33.9)	304	(41.0)
Race and Ethnicity																
Hispanic of Any Race	232	(19.3)	13.6	(9.2)	43.1	(35.2)	3.5	(3.8)	126	(18.8)	56	(17.6)	54	(22.0)	126	(17.0)
Non-Hispanic Asian	22	(1.8)	16.3	(9.3)	37.2	(41.4)	2.5	(2.5)	13	(1.9)	6	(1.9)	6	(2.4)	14	(1.9)
Non-Hispanic Black	35	(2.9)	11.0	(7.4)	21.9	(19.9)	2.3	(2.4)	16	(2.4)	10	(3.1)	2	(0.80)	25	(3.4)
Non-Hispanic White	871	(72.6)	13.6	(8.9)	38.0	(32.1)	2.8	(3.3)	488	(72.6)	241	(75.8)	172	(70.2)	554	(74.8)
Other	17	(1.4)	18.5	(11)	43.4	(42.2)	3.4	(4.6)	12	(1.8)	2	(0.60)	3	(1.2)	11	(1.5)
Cohort locations																
Phoenix, AZ	236	(19.7)	14.6	(9.3)	41.0	(34.7)	3.3	(3.5)	138	(20.5)	66	(20.8)	46	(18.8)	154	(20.8)
Tucson, AZ	318	(26.5)	14.3	(8.9)	40.0	(34.8)	3.0	(3.6)	176	(26.2)	89	(28.0)	71	(29.0)	188	(25.4)
Miami, FL	138	(11.5)	11.9	(9.0)	48.9	(36.2)	3.2	(3.6)	78	(11.6)	32	(10.1)	37	(15.1)	79	(10.7)
Duluth, MN	134	(11.2)	13.8	(10.1)	35.9	(32.1)	2.5	(3.5)	75	(11.2)	42	(13.2)	24	(9.8)	93	(12.6)
Portland, OR	90	(7.5)	13.8	(7.6)	38.4	(28.2)	2.9	(2.8)	56	(8.3)	23	(7.2)	19	(7.8)	60	(8.1)
Temple, TX	97	(8.1)	12.3	(8.7)	26.2	(22.4)	2.6	(2.2)	44	(6.5)	18	(5.7)	22	(9.0)	40	(5.4)
Salt Lake City, UT	186	(15.5)	13.0	(8.0)	35.7	(30.0)	2.8	(3.2)	105	(15.6)	48	(15.1)	26	(10.6)	127	(17.1)
Occupation [d]																
Primary HCP	187	(15.6)	12.7	(7.6)	30.8	(27.1)	1.9	(2.3)	92	(13.7)	64	(20.1)	24	(9.8)	132	(17.8)
Nurses and other allied HCP	348	(29.0)	14.2	(9.5)	37	(29.5)	3.5	(3.7)	194	(28.9)	91	(28.6)	84	(34.3)	197	(26.6)
First Responders	336	(28.0)	13.5	(9.0)	53	(36.7)	2.8	(3.4)	195	(29.0)	77	(24.2)	76	(31.0)	197	(26.6)

Essential and other frontline	328	(27.4)	13.8	(8.9)	31.7	(31.3)	3.2	(3.3)	191	(28.4)	86	(27.0)	61	(24.9)	215	(29.0)
Medical History																
Chronic Condition [e]																
None	854	(71.2)	13.4	(9.0)	38.8	(32.8)	2.8	(3.3)	476	(70.8)	230	(72.3)	159	(64.9)	544	(73.4)
1 or more	329	(27.4)	14.2	(8.8)	38.7	(33.3)	3.5	(3.6)	188	(28.0)	83	(26.1)	81	(33.1)	190	(25.6)
Number of medications																
None	589	(49.1)	13.0	(8.8)	37.5	(31.7)	2.5	(3.0)	314	(46.7)	169	(53.1)	94	(38.4)	385	(74.4)
1	250	(20.9)	13.7	(8.5)	36.9	(31.6)	2.9	(3.1)	143	(21.3)	65	(20.4)	45	(18.4)	164	(74.4)
2 or more	332	(27.7)	14.8	(9.4)	42.6	(35.7)	3.9	(3.9)	198	(29.5)	77	(24.2)	99	(40.4)	176	(74.4)
Self-Rated Health																
Excellent/Very good	853	(71.1)	13.1	(8.5)	37.7	(32.2)	2.7	(3.2)	482	(71.7)	231	(72.6)	158	(64.5)	553	(74.6)
Good/Fair/Poor	346	(28.9)	15.1	(9.8)	41.5	(34.6)	3.7	(3.8)	190	(28.3)	87	(27.4)	87	(35.5)	188	(25.4)

Abbreviations: Number (No.), Column (Col.), Standard deviation (SD), Healthcare personnel (HCP), Messenger RNA (mRNA), HEROES (the Arizona Healthcare, Emergency Response, and Other Essential Workers Surveillance Study), RECOVER (Research on the Epidemiology of SARS-CoV-2 in Essential Response Personnel)

a. Febrile illness defined as self-reported fever, feverishness, chills, or measured temperature >38 degrees Celsius

b. Afebrile defined as anyone who didn't report fever or chills in surveys

c. Among 207 participants that received medical care, 27 sought care from the emergency room and 8 were hospitalized.

d. Primary HCP included physicians, physician assistants, nurse practitioners, and dentists; allied HCP included nurses, therapists, technicians, medical assistants, orderlies, and all others providing clinical support in inpatient and outpatient settings; other essential and frontline workers included teachers and hospitality, delivery, and retail workers, as well as all other occupations that require routine close contact with the public, customers, or coworkers; first responders included firefighters, law enforcement, corrections officers, and emergency medical technicians.

e. Chronic condition included asthma, chronic lung disease, cancer, diabetes, heart disease or condition, immunosuppression, kidney disease, liver disease, neurologic or neuromuscular disease, autoimmune disease.

eTable 4. Participant characteristics by viral RNA load and plaque forming units among SARS-CoV-2 infected adults

	Viral RNA Load log10 copies/μL				Plaque forming units log10 copies/mL			
Characteristics	No. Infections		Mean	SD	No. Quantified/No. Tested [a]		Mean	SD
Total	1199		3.5	(1.7)	125/157		4.1	(1.6)
Demographics								
Age (Years)								
18 - 49	878		3.5	(1.7)	92/116		4.2	(1.6)
≥50	321		3.6	(1.7)	33/41		3.9	(1.3)
Sex								
Female	714		3.4	(1.7)	69/91		4.2	(1.6)
Male	483		3.6	(1.8)	55/65		4.0	(1.5)
Race and Ethnicity								
Hispanic of Any Race	232		3.5	(1.7)	23/27		4.2	(1.8)
Non-Hispanic Asian	22		3.3	(1.9)	1/2		2.7	(-)
Non-Hispanic Black	35		3.0	(1.8)	0/1		-	(-)
Non-Hispanic White	871		3.5	(1.7)	94/120		4.2	(1.5)
Other	17		4.2	(1.6)	3/3		5.5	(1.1)
HEROES-RECOVER Cohort locations								
Phoenix, AZ	236		3.7	(1.6)	25/33		4.0	(1.5)
Tucson, AZ	318		3.5	(1.7)	20/25		3.9	(1.5)
Miami, FL	138		3.5	(1.7)	14/18		4.3	(1.9)
Duluth, MN	134		3.6	(1.6)	18/21		4.0	(1.6)
Portland, OR	90		3.7	(1.7)	12/15		4.9	(1.5)
Temple, TX	97		2.8	(1.9)	12/15		4.6	(1.2)
Salt Lake City, UT	186		3.5	(1.8)	24/30		3.9	(1.6)
Occupation [b]								
Primary HCP	187		3.5	(1.7)	28/35		3.6	(1.5)
Nurses and other allied HCP	348		3.3	(1.8)	31/39		4.5	(1.6)
First Responders	336		3.7	(1.7)	35/44		4.1	(1.5)
Essential and other frontline	328		3.5	(1.7)	31/39		4.2	(1.5)

Medical History								
Chronic Condition [c]								
None	854		3.5	(1.7)	90/108		4.2	(1.6)
1 or more	329		3.6	(1.8)	34/48		4.0	(1.5)
Number of medications								
None	589		3.5	(1.7)	51/64		4.1	(1.5)
1	250		3.5	(1.7)	27/32		4.4	(1.5)
2 or more	332		3.6	(1.8)	44/57		4.0	(1.7)
Self-Rated Health								
Excellent/Very good	853		3.5	(1.7)	94/112		4.1	(1.5)
Good/Fair/Poor	346		3.5	(1.8)	31/45		4.3	(1.6)

Abbreviations: Standard Deviation (SD), Healthcare personnel (HCP), Number (No.)

[a] Number quantified includes all positive SARS-CoV-2 specimens with > 20 copies/mL

[b] Primary HCP included physicians, physician assistants, nurse practitioners, and dentists; allied health care personnel included nurses, therapists, technicians, medical assistants, orderlies, and all others providing clinical support in inpatient or outpatient settings; first responders included firefighters, law enforcement, corrections officers, and emergency medical technicians; and other essential and frontline workers included teachers and hospitality, delivery, and retail workers, as well as all other occupations that require routine close contact with the public, customers, or coworkers.

[c] Chronic condition included asthma, chronic lung disease, cancer, diabetes, heart disease or condition, immunosuppression, kidney disease, liver disease, neurologic or neuromuscular disease, autoimmune disease.

eTable 5. Participant and illness characteristics by viral RNA load and plaque forming units.

		Viral RNA load (log10 copies/μL)					Plaque forming units (log10 copies/mL)				
	No. Infections	Mean	SD	Mean difference	(95% CI)		No. Quantified/ No. Tested [a]	Mean	SD	Mean difference	(95% CI)
SARS-CoV-2 Lineage											
Origin	168	3.8	(1.7)	**-1.5**	**(-2.7, -0.2)**		9/13	3.5	(1.0)	-	(-)
Delta variant	288	4.0	(1.7)	0.20	(-0.2, 0.6)		116/144	4.2	(1.6)	-	(-)
Omicron variant	743	3.2	(1.7)	ref	(-)		-	-	(-)	-	(-)
Origin Strain Infection Characteristics											
Symptomatic COVID-19 [b]											
COVID-19 symptoms	147	3.9	(1.6)	0.66	(-0.1, 1.5)		8/12	3.5	(1.1)	1.5	(-2.6, 5.6)
Asymptomatic	19	3.1	(1.8)	ref	(-)		1/1	3.0	(-)	ref	(-)
Fever or chills [b,c]											
No, Mean (SD)	42	3.9	(1.9)	ref	(-)		2/5	2.8	(2.2)	ref	(-)
Yes, Mean (SD)	103	4.0	(1.6)	0.14	(-0.5, 0.7)		6/7	3.7	(0.7)	4.8	(-0.7, 10.4)
Received medical care [b,d,e]											
No	90	4.0	(1.6)	ref	(-)		5/8	3.4	(1.2)	ref	(-)
Yes	54	3.9	(1.7)	0.11	(-0.5, 0.7)		3/4	3.7	(1.0)	-1.9	(-6.0, 2.2)
Total number of symptoms [b,f]											
0-2	12	3.8	(1.8)	ref	(-)		0/1	-	(-)	-	(-)
3-7	58	3.7	(1.8)	0.23	(-0.9, 1.3)		5/8	3.3	(1.3)	ref	(-)
≥8	75	4.1	(1.5)	0.66	(-0.4, 1.7)		3/3	3.8	(0.4)	1.7	(-1.8, 5.2)
Total days of symptoms [b]											
0-6	17	3.9	(1.3)	ref	(-)		1/3	4.3	(-)	ref	(-)
7-13	59	3.7	(1.8)	-0.61	(-1.5, 0.3)		3/3	2.9	(1.4)	-1.7	(-9.8, 6.3)
≥14	71	4.1	(1.6)	-0.02	(-0.9, 0.9)		4/6	3.8	(0.80)	-0.60	(-7.3, 6.1)
Days spent sick in bed [b,g]											
0	21	3.8	(1.8)	ref	(-)		1/3	1.3	(-)	ref	(-)
1-2	29	4.0	(1.7)	0.34	(-0.6, 1.2)		2/2	3.7	(1.0)	3.8	(-12.2, 19.7)
≥3	73	4.0	(1.6)	0.54	(-0.3, 1.3)		4/5	3.9	(0.4)	-3.3	(-12.3, 18.8)
Hours of missed work [g,h]											
0-15	32	3.4	(1.8)	ref	(-)		2/4	3.7	(1.0)	ref	(-)
16-47	18	4.0	(1.5)	0.57	(-0.3, 1.5)		0/0	-	(-)	-	(-)
≥48	73	4.3	(1.4)	**0.99**	**(0.3, 1.6)**		5/6	3.4	(1.2)	-1.2	(-6.7, 4.4)

Delta Variant Infection Characteristics											
	No. Infections	Mean	SD	Mean difference	(95% CI)		No. Quantified/ No. Tested [a]	Mean	SD	Mean difference	(95% CI)
Symptomatic COVID-19 [b]											
COVID-19 symptoms	260	4.1	(1.6)	**1.6**	**(0.9, 2.3)**		109/134	4.3	(1.6)	**2.0**	**(0.5, 3.4)**
Asymptomatic	25	2.6	(1.8)	ref	(-)		6/9	3.0	(1.0)	ref	(-)
Fever or chills [b,c]											
No, Mean (SD)	65	3.8	(1.6)	ref	(-)		26/30	3.9	(1.6)	ref	(-)
Yes, Mean (SD)	182	4.3	(1.6)	**0.55**	**(0.1, 1.0)**		76/95	4.4	(1.6)	0.32	(-0.4, 1.0)
Received medical care [b,i,j]											
No	159	4.2	(1.5)	ref	(-)		71/85	4.2	(1.6)	ref	()
Yes	88	4.0	(1.7)	-0.34	(-0.8, 0.1)		32/42	4.5	(1.6)	0.30	(-0.3, 1.0)
Total number of symptoms [b,f]											
0-2	25	3.3	(2.0)	ref	(-)		9/11	4.1	(2.2)	ref	(-)
3-7	88	4.1	(1.5)	**0.79**	**(0.1, 1.5)**		33/43	3.9	(1.5)	-0.11	(-1.3, 1.0)
≥8	136	4.4	(1.5)	**1.0**	**(0.3, 1.7)**		61/73	4.5	(1.5)	0.16	(-0.9, 1.2)
Total days of symptoms [b]											
0-6	32	3.6	(1.8)	ref	(-)		12/14	3.9	(1.9)	ref	(-)
7-13	98	4.1	(1.6)	0.37	(-0.3, 1.0)		46/57	4.2	(1.6)	0.88	(-0.2, 1.9)
≥14	130	4.3	(1.5)	**0.65**	**(0.0, 1.3)**		51/63	4.4	(1.6)	**1.1**	**(0.1, 2.1)**
Days spent sick in bed [b,g]											
0	53	3.9	(1.6)	ref	(-)		20/26	3.9	(1.6)	ref	(-)
0-2	33	4.1	(1.8)	0.04	(-0.6, 0.7)		15/16	4.4	(1.3)	0.71	(-0.3, 1.8)
≥3	117	4.3	(1.5)	0.31	(-0.2, 0.8)		53/66	4.4	(1.6)	0.17	(-0.6, 1.0)
Hours of missed work [g,h]											
0-15	35	4.0	(1.8)	ref	(-)		14/18	4.4	(1.6)	ref	(-)
16-47	55	4.0	(1.6)	-0.11	(-0.8, 0.6)		27/31	3.9	(1.6)	-0.50	(-1.6, 0.5)
≥48	113	4.3	(1.5)	0.13	(-0.5, 0.7)		47/59	4.4	(1.6)	-0.30	(-1.3, 0.6)
Omicron Variant Infection Characteristics											
	No. Infections	Mean	SD	Mean difference	(95% CI)		No. Quantified/ No. Tested [a]	Mean	SD	Mean difference	(95% CI)
Symptomatic COVID-19 [c]											
COVID-19 symptoms	628	3.4	(1.6)	**1.2**	**(0.8, 1.6)**		-	-	(-)	-	(-)

Asymptomatic	96	2.3	(1.8)	ref	(-)		-	-	(-)	-	(-)
Fever or chills [b,c]											
No	210	3.1	(1.6)	ref	(-)		-	-	(-)	-	(-)
Yes	387	3.5	(1.6)	**0.38**	**(0.1, 0.7)**		-	-	(-)	-	(-)
Received medical care [b,k]											
No	492	3.4	(1.6)	ref	(-)		-	-	(-)	-	(-)
Yes	102	3.2	(1.7)	-0.35	(-0.7, 0.0)		-	-	(-)	-	(-)
Total number of symptoms [b,f]											
0-2	72	2.8	(1.8)	ref	(-)		-	-	(-)	-	(-)
3-7	291	3.4	(1.6)	**0.64**	**(0.2, 1.1)**		-	-	(-)	-	(-)
≥8	238	3.5	(1.6)	**0.83**	**(0.4, 1.3)**		-	-	(-)	-	(-)
Total days of symptoms [b]											
0-6	164	3.1	(1.7)	ref	(-)		-	-	(-)	-	(-)
7-13	273	3.4	(1.6)	**0.37**	**(0.0, 0.7)**		-	-	(-)	-	(-)
≥14	191	3.5	(1.6)	**0.52**	**(0.2, 0.9)**		-	-	(-)	-	(-)
Days spent sick in bed [b,g]											
0	162	3.2	(1.7)	ref	(-)		-	-	(-)	-	(-)
0-2	185	3.5	(1.5)	0.41	(0.0, 0.8)		-	-	(-)	-	(-)
≥3	189	3.4	(1.6)	0.33	(-0.0, 0.7)		-	-	(-)	-	(-)
Hours of missed work [g,h]											
0-15	175	3.3	(1.6)	ref	(-)		-	-	(-)	-	(-)
16-47	224	3.3	(1.7)	-0.10	(-0.5, 0.3)		-	-	(-)	-	(-)
≥48	137	3.5	(1.6)	0.21	(-0.2, 0.6)		-	-	(-)	-	(-)

Abbreviations: Number (No.), Column (Col.), Standard deviation (SD), Confidence interval (CI)

[a] Number detected includes all positive SARS-CoV-2 specimens with > 20 copies/mL.

[b] Mean differences estimated using linear regression and adjusted for index date to swab collection, reported number of medications and race and ethnicity. Index date is defined as the date of illness onset or the date of specimen collection if the infection was asymptomatic

[c] Fever or chills defined as self-reported fever, feverishness, chills, or measured temperature >38 degrees Celsius.

[d] Among those that received medical care and had quantified viral RNA load, 7 sought care from the emergency department and 3 were hospitalized.

[e] Among those that received medical care and had quantified plaque forming units, none were hospitalized or visited the emergency department

[f] List of symptoms include the following: Fever or feeling feverish, chills, dry cough, cough that produces phlegm or sputum, sore throat, runny nose or nasal congestion, shortness of breath or difficulty breathing, muscle or body aches, joint pain,

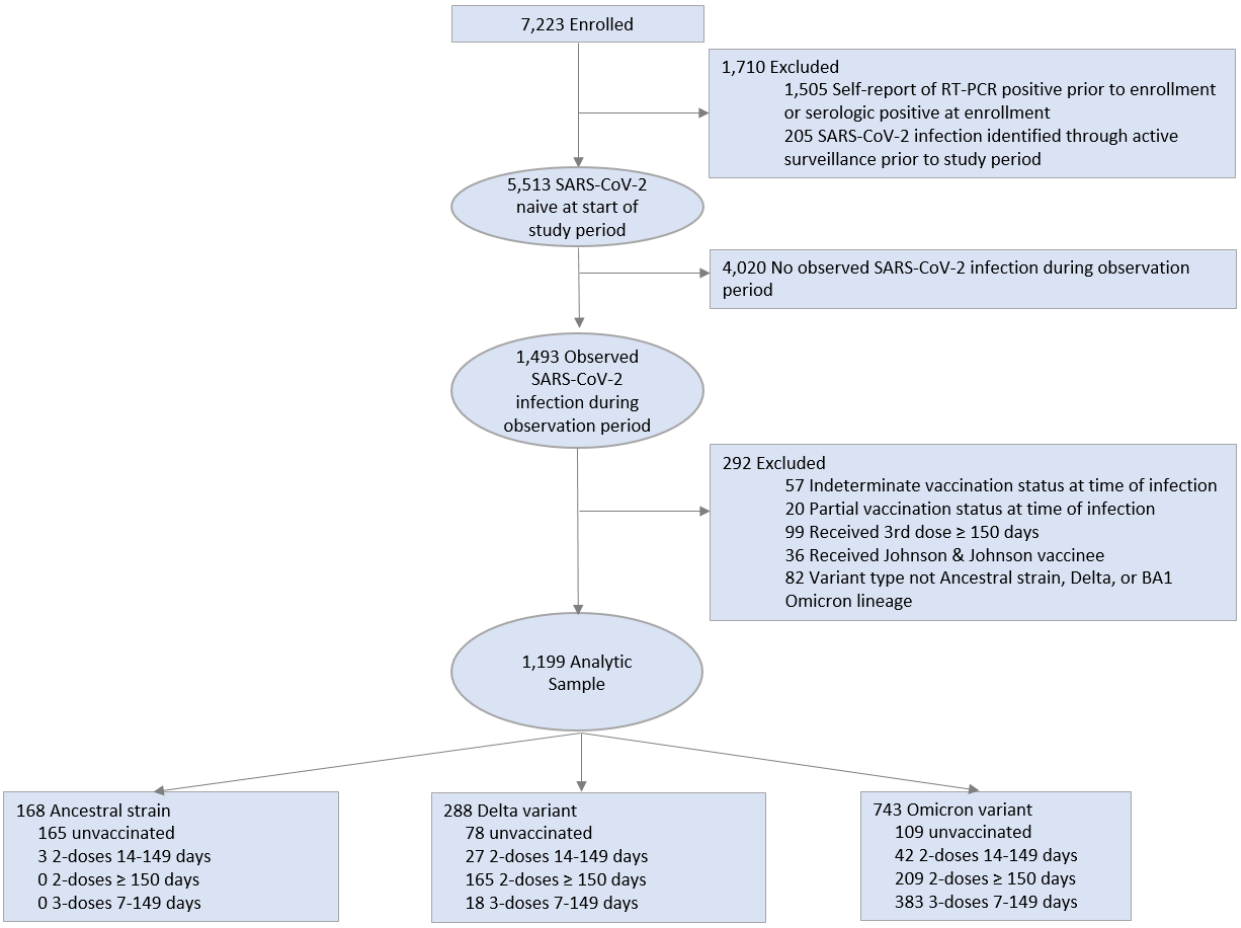
	abdominal pain, nausea or queasy feeling that you may vomit, vomiting, diarrhea, headache, fatigue, rash, discoloration or blistering of fingers or toes, eye redness, loss of smell or taste.		
	[g] Assessed among participants who reported COVID-19 symptoms and responded to questions on days spent sick in bed and hours of missed work.		
	[h] Mean differences estimated using linear regression and adjusted for index date to swab collection, reported number of medications, race and ethnicity, and occupation category. Index date is defined as the date of illness onset or the date of specimen collection if the infection was asymptomatic		
	[i] Among those that received medical care and had quantified viral RNA load, 15 sought care from the emergency department and 4 were hospitalized.		
	[j] Among those that received medical care and had quantified plaque forming units, 1 was hospitalized and 8 sought care from the emergency department.		
	[k] Among those that received medical care and had quantified viral RNA load, 3 sought care from the emergency department.		

eTable 6. Time course of infection by virus strain and symptomology

	N participants	N samples	Estimated Mean Viral RNA load	Mean Difference	95% Credible interval	Estimated peak viral RNA load[a]	95% Credible interval
SARS-CoV-2 Lineage							
Origin strains	63	220	1.7	0.3	(0.1, 0.5)	4.4	(4.2, 4.6)
Delta variant	96	323	1.7	0.2	(0.0, 0.4)	4.4	(4.2, 4.6)
Omicron variant	248	850	1.4	(ref)		4.2	(4.0, 4.3)
Symptoms Associated with SARS-CoV-2							
Symptomatic COVID-19 [a]	381	1308	1.5	0.7	(0.4, 1.1)	4.5	(4.4, 4.6)
Asymptomatic [b]	20	66	0.70	(ref)		3.8	(3.4, 4.2)

eFigures

eFigure 1. Flow diagram



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