Supplementary Appendix

Supplement to: Boucau J, Marino C, Regan J, et al. Duration of shedding of culturable virus in SARS-CoV-2 omicron (BA.1) infection. N Engl J Med. DOI: 10.1056/NEJMc2202092

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

Table of Contents

Methods

Study Participants

Non-hospitalized individuals with positive SARS-CoV-2 PCR tests in the Mass General Brigham medical system were recruited (Supplementary Figure S4). Adults over 18 years of age with a positive test within the previous four days in the medical health record were recruited, irrespective of indication for testing (i.e. for symptomatic disease, contact tracing, or work or pre-operative screening). The study team extracted an automated list of positive COVID-19 tests from the electronic medical record. The list was restricted to individuals residing in the study catchment area- a 40-mile radius around the hospital established by the need to conduct home-based specimen collection. At the start of each week, patients with positive results were stratified by vaccination status and ranked by recency of positive result with the goal of recruiting 2-3 new participants per week, which was the maximum capacity of the team to conduct ambulatory specimen collection. For those who consented to participation, we conducted home visits three times weekly for two weeks or until negative PCR testing. At each visit, we obtained self-collected nasal swabs in viral transport media, which were transported to the laboratory within four hours of collection, aliquoted and frozen at -80^{o}C until future testing. Symptoms, date of onset, and severity were recorded at each specimen collection. Symptomatic infections were defined as those with COVID-19-related symptoms at any point during the observation period. Individuals treated with monoclonal antibodies were excluded from this analysis, based on data demonstrating that they impact the rate of culture conversion[1].

Viral load Quantification

Viral load quantification was carried out as previously reported[2]. Briefly, nasal swab fluids were centrifuged at 21,000 x g for 2 hours at 4°C to pellet virions. 750 µL TRIzol-LS™ Reagent (ThermoFisher) was then added to the pellets, and samples were subsequently incubated on ice for 10 minutes. 200 µL of chloroform (MilliporeSigma) was added to each sample, and the

resulting mixtures were then vortexed and centrifuged at 21,000 x g for 15 minutes at 4°C. The clear aqueous layer was collected and combined with an equal volume of isopropanol (Sigma), 1.5 µL GlycoBlue™ Coprecipitant (ThermoFisher) and 100 µL 3M Sodium Acetate (Life Technologies); the resulting mixtures were briefly shaken and then incubated on dry ice. Samples were centrifuged at 21,000 x g for 45 minutes at 4°C to yield RNA pellets, which were washed with cold 70% ethanol before being resuspended in 50 µL DEPC-treated water (ThermoFisher). Using the US CDC 2019-nCoV_N1 primer and probe set (IDT) (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html) and N1 qPCR standards in 16-fold dilutions to generate standard curves, SARS-CoV-2 viral RNA was quantified. Each reaction consisted of extracted RNA, 1X TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher), forward and reverse primers, and the probe. Each sample was run in triplicate, and all plates contained two non-template control (NTC) wells. Positive and negative controls were run alongside all samples. To ensure appropriate sample quality, the Importin-8 (IPO8) housekeeping gene RNA level was quantified. The efficiency of the RNA extraction and qPCR amplification was assessed by quantifying the internal virion control $RCAS^3$ RNA level after spiking this viral mixture into each sample.

SARS-CoV-2 culture

Viral culture was performed in the BSL3 laboratory of the Ragon Institute of MGH, MIT, and Harvard as previously reported [2]. Briefly, Vero-E6 cells (American Type Culture Collection) maintained in DMEM (Corning) supplemented with HEPES (Corning), 1X Penicillin 100IU/mL/Streptomycin 100 ug/mL (Corning), 1X Glutamine (Glutamax, ThermoFisher Scientific), and 10% Fetal Bovine serum (FBS) (Sigma) using Trypsin-EDTA (Fisher Scientific) were detached and seeded at 20,000 cells per well in 96w plates 16-20 hours before infection. Specimens were thawed on ice and filtered through a Spin-X 0.45um filter (Corning) at 10,000 x g for 5min. 25ul of the undiluted filtrate was added to four wells of a 96w plate and serial diluted

(1:5) across half of the plate in media containing 5ug/mL of polybrene (Santa Cruz Biotechnology). Plates were centrifuged for 1 hour at 2000 x g at 37C. The positive control SARS-CoV-2 isolate USA-WA1/2020 strain (BEI Resources) was used in parallel for all assays. Plates were observed with a light microscope 7 days post-infection and documented wells with CPE. Supernatant of wells was harvested for RNA isolation using a QIAamp Viral RNA Mini kit (QIAGEN) for confirmation of the viral sequence. For each variant, we selected four specimens with viral loads between 5.2log and 6.5log, two with positive culture and two with negative culture results and repeated the TCID50 protocol using Vero-E6 and A549-Ace2 (BEI Resources) cell lines. In addition to the spinfection protocol described above, we included a set of plates that were not centrifuged after addition of the filtered clinical specimens but were incubated at 37C and 5% CO2 instead. Plates were then scored for CPE daily on days 3-7 post-inoculation. CPE was only observed following the spinfection protocol, and the results of the initial TCID50 experiments were confirmed. For the specimens showing CPE, CPE was observed at similar or earlier timepoints in Vero-E6 cells than in A549-ACE2 cells, and TCID50 on day 7 was similar between the two cell lines.

SARS-CoV-2 Whole Genome sequencing

Whole genome sequencing was performed as previously described[2] following the Illumina COVIDSeq Test protocol. Libraries were constructed using the Illumina Nextera XT Library Prep Kit, then pooled and quantified using a Qubit High Sensitivity dsDNA kit (Invitrogen, Waltham, MA, USA). Genomic sequencing was performed on an Illumina NextSeq 2000, Illumina NextSeq 550, or Illumina NovaSeq SP instrument. Sequences with an assembly length greater than 24,000 base pairs were considered complete genomes, and those sequences were assigned a Pango lineage using the most up-to-date version of pangoLEARN assignment algorithm v2.4.2[4]. All sequences were deposited to GenBank and GISAID. The samples were submitted to NCBI with Bioproject Accession numbers PRJNA715749 or PRJNA759255.

SARS-CoV-2 TaqPath RT-PCR Assay

Starting with Participant 200, samples were tested for spike gene target failure (SGTF) as an additional genotyping method of detecting Omicron cases following the TaqPath COVID-19 Combo Kit protocol (Thermo Fisher Scientific, Waltham, MA, USA). Nucleic acid was extracted using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit on a Thermo KingFisher Flex purification system (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription PCR (RT-PCR) was conducted on extracted samples using the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems, Waltham, MA, USA), then analyzed for the presence of SARS-CoV-2 on ORF1ab, N gene, and S gene targets. SGTF was determined by amplification of SARS-CoV-2 for the ORF1ab and N gene targets with CT values <36 along with the lack of amplification for the S gene target.

SARS-CoV-2 Spike gene amplification

Spike gene amplification was also performed as previously described[2] to determine variant types for specimens with low viral load when whole genome sequencing was unsuccessful. cDNA synthesis was synthesized using Superscript IV reverse transcriptase (Invitrogen, Waltham, MA, USA) as per manufacturer's protocols. cDNA amplification was performed using *in-house* designed primer sets that targeted codon 1-814 of the spike gene. PCR products were pooled for Illumina library construction using the Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA). Raw sequence data was analyzed with PASeq v1.4 (https://www.paseq.org). Amino acid variants were identified at the codon level with perl code and the resulting variant file was used to determine SARS-CoV-2 variant type using Nextclade version 1.13.1 (https://doi.org/10.21105/joss.03773).

Statistical methods

We summarized demographic and clinical characteristics for individuals with delta and omicron variant infection and compared characteristics by sub-group with chi-squared testing for categorical variables and non-parametric testing for continuous variables. We graphically depicted viral decay by variant with a scatter plot and median of viral load over time since the index positive PCR test. To determine whether variant type or vaccination status was associated with virologic decay, we used the Kaplan-Meyer method to estimate the survivor function for two outcomes of interest: 1) time to conversion to negative PCR and 2) time to conversion to viral culture negative. For both outcomes we conducted two survival analyses defining the origin of observation as either 1) the date of the first positive PCR test or 2) the earliest of the date of symptom onset or the first positive PCR test. We defined the exit as the first negative test (PCR or culture) after the last positive result. Individuals who had a positive PCR or culture on the final day of observation were censored as positive. For both outcomes, we constructed Kaplan-Meier curves of survival by variant and vaccination status. We categorized vaccination status as unvaccinated, vaccinated, for those who had received two COVID-19 vaccinations (or a single dose of the Johnson & Johnson/Janssen vaccine) at least 14 days prior to enrollment, and boosted for those who had received three COVID-19 vaccinations (or a second dose of the Johnson & Johnson/Janssen vaccine) at least 14 days prior to enrollment. We then fitted Cox proportional hazards models with both outcomes, and age, sex, vaccination status, and variant of infection as predictors.

Study approval

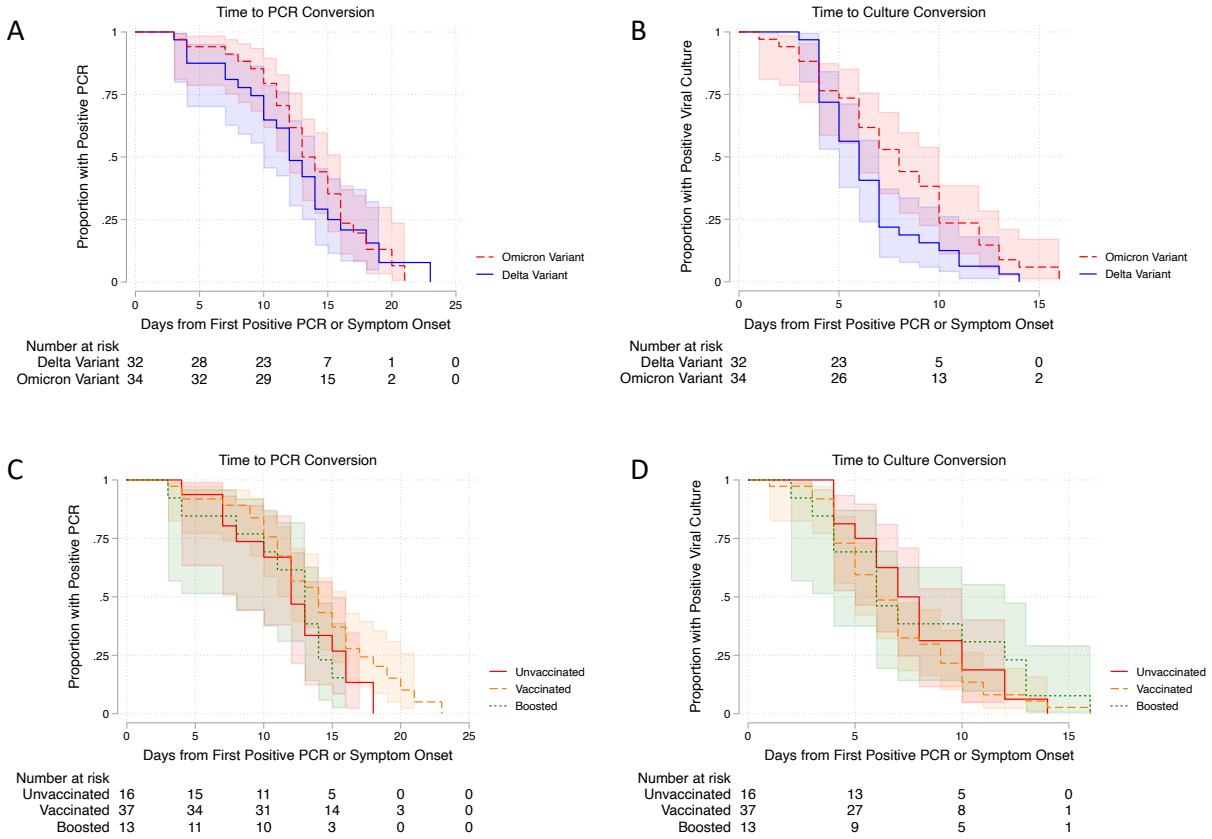
Study procedures were approved by the Human Subjects Institutional Review Board and the Institutional Biosafety Committee at Mass General Brigham. All participants gave verbal informed consent, as written consent was waived by the review committee based on the risk to benefit ratio of requiring in-person interactions for an observational study of COVID-19.

Acknowledgements

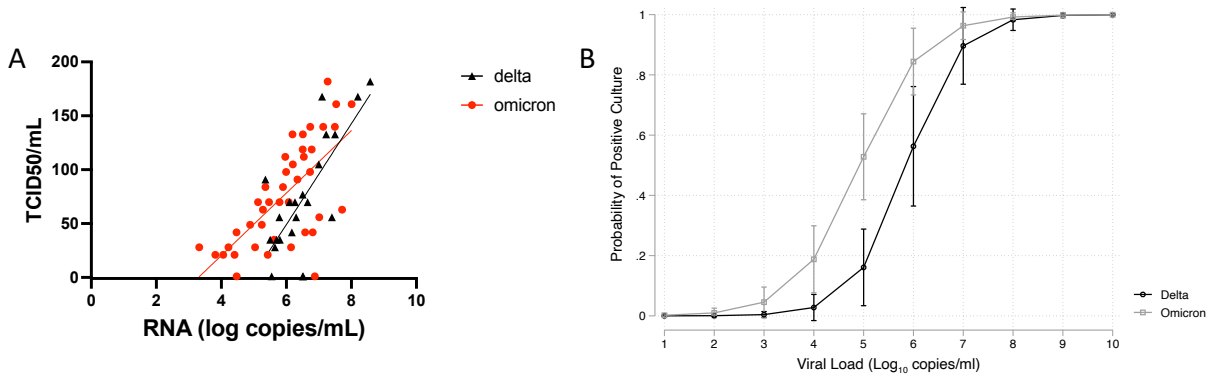
The authors would like to thank study participants for willingness to engage in the study.

Author Contributions

J.B., M.G.B., J.M.V., J.Z.L, J.E.L, M.J.S, and A.K.B. designed the work. J.B., C.M., J.R., R.U., M.C.C., J.P.F., G.C., A.M.S., J.M., M.Y.L., A.S., T.L., A.K., M.M., Y.L., R.F.G., Z.R., S.L.I., G.C.C. and T.D.V. performed the work. J.B., J.R., R.U., M.C.C., J.P.F, Y.L., J.Z.L, J.E.L, M.J.S, and A.K.B. analyzed and reported the work.



Supplementary Figure S1. Kaplan-Meier survival curves demonstrating time from the earlier of symptom onset or initial positive PCR to negative PCR by viral variant (A) and vaccination status (C) and time from the earlier of symptom onset or initial positive PCR to negative viral culture by viral variant (B) and vaccination status (D). Shaded areas indicate 95% confidence intervals.



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179 **Supplementary Figure S2.** A. Relationship between viral load and TCID50 for Omicron and Delta
180 infections. Each point represents a nasal swab specimen collected from an individual with Delta
181 (triangle) or Omicron (circle) variant infection. B. Predicted probability of a positive viral culture
182 by viral load for delta (black line) and Omicron (gray line) infection. Curves were derived from
183 post-regression margin from logistic regression model of viral load and variant on viral culture
184 results.
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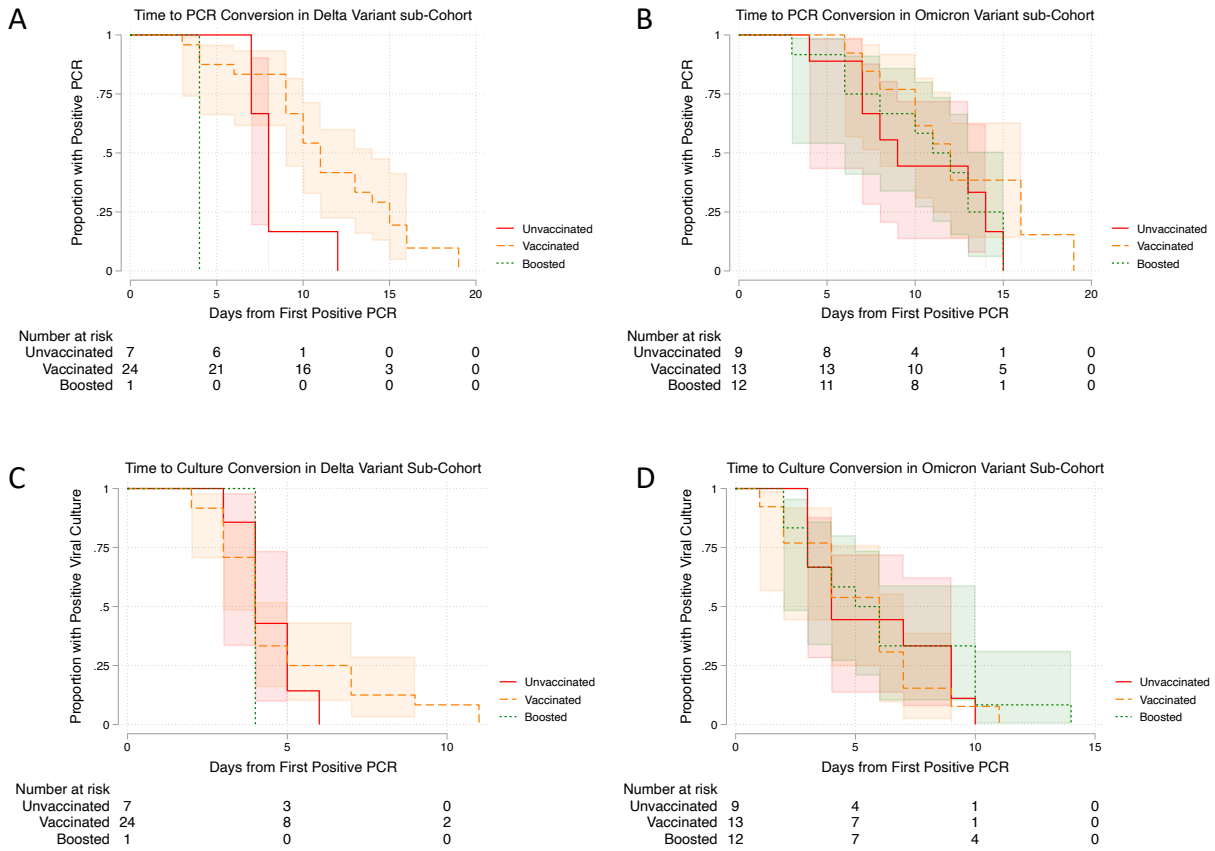
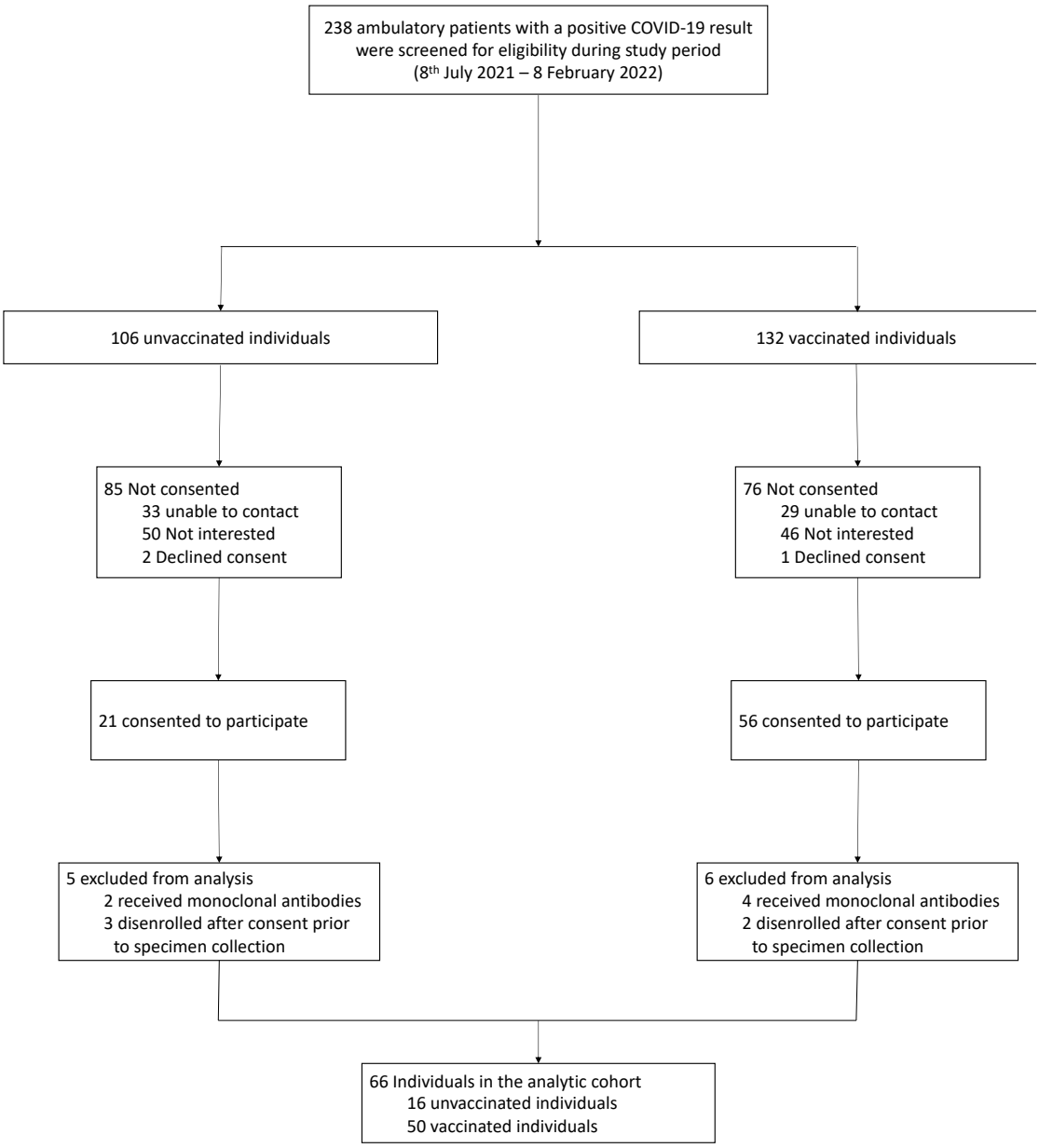


Supplementary Figure S3. (A-B) Kaplan-Meier survival curves demonstrating time from initial positive PCR to negative PCR by vaccination status for individuals with Delta variant infection (A) and Omicron variant infection (B). (C-D) Time from initial positive PCR to negative viral culture by vaccination status for Delta variant infection (C) and Omicron variant infection (D). Shaded areas indicate 95% confidence intervals.



Supplementary Figure S4. Schema of participant recruitment.

197 **Table S1**. Cohort characteristics

	Omicron variant infection (n=34)	**Delta variant infection (n=32)**
Female, n (%)	24 (71%)	21 (66%)
Age, mean (SD)	42 (15)	40 (16)
Vaccination status, n (%)		
Unvaccinated	9 (26%)	7 (22%)
Vaccinated	13 (38%)	24 (75%)
Boosted	12 (35%)	1 (3%)
Days since last vaccination, mean (SD)	143 (119)	199 (72)
Symptomatic infection, n (%)	34 (100%)	31 (97%)
Nasal swabs collected, n (%)		
7	29 (86%)	25 (78%)
6	4 (12%)	5 (16%)
≤5	1 (3%)	2 (6%)
Days from index PCR to first study specimen (median, IQR)	2 (2-3)	3 (2-4)

198

Table S2. Demographics of study participants relative to individuals with COVID-19 infection reported in the state of Massachusetts (cumulative cases as of 4/21/22).

	COVID-19 Cases in Massachusetts (through 4/21/22) n = 1,598,541	**Study participants** (n = 66)	**Comments**
Age			
0-18	104,044 (31%)	0 (0%)	Children were not included in the study. The age of participants was similar to the age of adults infected with COVID-19 in the general population, although no participants were 80yo or older.
19-29	31,994 (12%)	19 (29%)	
30-39	30,499 (11%)	15 (23%)	
40-49	26,553 (10%)	12 (18%)	
50-59	22,713 (9%)	12 (18%)	
60-69	17,004 (6%)	4 (6%)	
70-79	13,976 (5%)	4 (6%)	
80+	18,691 (7%)	0 (0%)	
Sex			
Male	809,030 (48%)	45 (68%)	Women were somewhat over-represented in the study population relative to the percent of COVID-19 cases in women in the general population.
Female	907,232 (52%)	21 (32%)	
Transgender	73 (<1%)	0 (0%)	
Unknown sex	16,821 (<1%)	0 (0%)	
Race/Ethnicity			
Asian, non-Hispanic	58,211 (3%)	5 (8%)	Individuals self-identifying as Asian, non-Hispanic, Black or African American, and White, non-Hispanic were all somewhat over-represented in the study cohort relative to COVID-19 cases in the general population. Individuals self-identifying as Hispanic were represented in a similar proportion to COVID-19 cases in the general population.
Black or African American, non-Hispanic)	102,043 (6%)	8 (12%)	
White, non-Hispanic	295,740 (17%)	38 (58%)	
Hispanic	650,043 (38%)	10 (15%)	
Other race, non-Hispanic	156,459 (9%)	0 (0%)	
American Indian/Alaskan Native, non-Hispanic	1591 (<1%)	0 (0%)	
Native Hawaiian/Pacific Islander, non-Hispanic	557 (<1%)	0 (0%)	
Unknown, refused or missing	468,512 (27)	4 (6%)	

State data extracted from the Massachusetts Department of Public Health publicly reported data https://www.mass.gov/info-details/covid-19-response-reporting#covid-19-interactive-data-dashboard-. Age, race, and ethnicity data for the study population was extracted from self-reported information in the medical record.

Table S3. Median time to PCR and culture conversion

	Days to confirmed PCR Conversion (median, IQR)	Days to Confirmed Culture Conversion (median, IQR)
Days from Index PCR		
Delta	10 (8-14)	4 (3-5)
Omicron	11 (8-15)	5 (3-9)
Days from Index PCR or Symptom Onset		
Delta	12 (9-15)	6 (4-7)
Omicron	13 (11-16)	8 (5-10)

209 **Table S4**. Cox proportional hazards model of time from first positive PCR test to PCR conversion

Covariate	Univariable Models Hazard Ratio (95%CI)	Multivariable Models Hazard Ratio (95%CI)
Age (10 years)	0.98 (0.82, 1.16)	0.98 (0.82, 1.17)
Sex		
Male	REF	REF
Female	1.14 (0.64, 2.04)	1.03 (0.57, 1.86)
Vaccination status		
Unvaccinated	REF	REF
Vaccinated	0.52 (0.27, 1.00)	0.44 (0.22, 0.90)
Boosted	0.77 (0.35, 1.71)	0.91 (0.39, 2.14)
Variant		
Delta	REF	REF
Omicron	0.86 (0.51, 1.44)	0.61 (0.33, 1.15)

210

211 **Table S5**. Cox proportional hazards model of time to culture conversion

Covariate	Univariable Models Hazard Ratio (95%CI)	Multivariable Models Hazard Ratio (95%CI)
Age	0.83 (0.70, 0.99)	0.84 (0.69, 1.02)
Sex		
Male	REF	REF
Female	1.24 (0.73, 2.10)	1.32 (0.75, 2.33)
Vaccination status		
Unvaccinated	REF	REF
Vaccinated	0.97 (0.54, 1.75)	1.07 (0.57, 2.00)
Boosted	0.72 (0.34, 1.55)	1.01 (0.43, 2.33)
Variant		
Delta	REF	REF
Omicron	0.76 (0.46, 1.24)	0.77 (0.44, 1.37)

212

Supplementary References

1. Boucau J, Chew KW, Choudhary M, et al. Monoclonal antibody treatment drives rapid culture conversion in SARS-CoV-2 infection. medRxiv 2021:2021.12.25.21268211.

2. Siedner MJ, Boucau J, Gilbert RF, et al. Duration of viral shedding and culture positivity with post-vaccination SARS-CoV-2 delta variant infections. JCI Insight 2021.

3. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2003;41:4531-6.

4. O'Toole A, Scher E, Underwood A, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. Virus Evol 2021;7:veab064.