

## Supplementary Appendix

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

# Supplementary Appendix

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The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the article.

## **Author contributions**

HNA and HC co-designed the study, performed the statistical analyses, and co-wrote the first draft of the article. LJA conceived and co-designed the study, led the statistical analyses, and co-wrote the first draft of the article. PT and MRH conducted the multiplex, RT-qPCR variant screening and viral genome sequencing. HY, FMB, and HAK conducted viral genome sequencing. All authors contributed to data collection and acquisition, database development, discussion and interpretation of the results, and to the writing of the manuscript. All authors have read and approved the final manuscript.

### **Competing interests**

Dr. Butt has received institutional grant funding from Gilead Sciences unrelated to the work presented in this paper. Otherwise we declare no competing interests.

## Section S1. Study population, data sources, and study design

This study was conducted in the resident population of Qatar, applying the test-negative, case-control study design<sup>1-3</sup> to investigate the protection afforded by prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in preventing reinfection with SARS-CoV-2 variants. Effectiveness of prior infection in preventing reinfection ( $PE_S$ ) was defined as the proportional reduction in susceptibility to infection among those with prior infection versus those without.<sup>3,4</sup> The test-negative methodology was recently developed and validated for the specific derivation of rigorous and robust estimates for SARS-CoV-2  $PE_S$ .<sup>3</sup>

COVID-19 laboratory testing, vaccination, clinical infection data, and related demographic details were extracted from the national, federated SARS-CoV-2 databases that include all polymerase chain reaction (PCR) testing, COVID-19 vaccinations, and COVID-19 hospitalizations and deaths in Qatar since the start of the pandemic, with no missing information on variables included in this study.

Every PCR test conducted in Qatar is classified based on the reason for testing (clinical symptoms, contact tracing, surveys or random testing campaigns, individual requests, routine healthcare testing, pre-travel, at port of entry, or other). Qatar has unusually young, diverse demographics, in that only 9% of its residents are  $\geq 50$  years of age, and 89% are expatriates from over 150 countries.<sup>5,6</sup> Nearly all individuals were vaccinated in Qatar, however, vaccinations performed elsewhere were still recorded in the health system at the port of entry upon arrival to Qatar per country requirements.

For estimation of  $PE_S$  against the Alpha<sup>7</sup> (B.1.1.7), Beta<sup>7</sup> (B.1.351), and Delta<sup>7</sup> (B.1.617.2) variants, cases (PCR-positive persons with genotyped variant infection) and controls (PCR-negative persons) identified between March 23, 2021 (start of positive samples' genotyping in

Qatar) and November 18, 2021 (prior to suspected introduction of the Omicron variant), were exact matched in a ratio of one-to-five by sex, 10-year age group, nationality, and calendar week of the PCR test (Figure S1 and Table S1). Infection with Alpha, Beta, or Delta variants was ascertained using real-time reverse-transcription PCR (RT-qPCR) genotyping of the positive clinical samples (Section S2).<sup>8,9</sup>

A similar methodology was applied to estimate  $PE_S$  against the Omicron<sup>7</sup> (B.1.1.529) variant. However, cases (PCR-positive persons with Omicron infection) and controls (PCR-negative persons) identified between December 23 and January 2, 2022, the time during which the Omicron epidemic wave was exponentially growing in Qatar, were exact matched in a ratio of one-to-three by sex, 10-year age group, nationality, and calendar day of the PCR test (rather than calendar week of the PCR test; Figure S2 and Table S2). A SARS-CoV-2 infection with the Omicron variant was proxied as an S-gene “target failure” case using the TaqPath COVID-19 Combo Kit platform (Thermo Fisher Scientific, USA<sup>10</sup>) applying the criterion of an RT-qPCR Ct value  $\leq 30$  for both the N and ORF1ab genes, but a negative outcome for the S gene.

Description of laboratory methods for the RT-qPCR testing and variant ascertainment are found in Section S2. All PCR testing was conducted at the Hamad Medical Corporation Central Laboratory or at Sidra Medicine Laboratory, following standardized protocols.

Matching of cases and controls was performed to control for known differences in the risk of exposure to SARS-CoV-2 infection in Qatar.<sup>6,11-14</sup> Only cases with an RT-qPCR cycle threshold (Ct) value  $\leq 30$  and individuals tested because of clinical suspicion, that is presence of symptoms compatible with a respiratory tract infection, were included in analysis. These criteria were applied to ensure that  $PE_S$  is estimated against reinfections with at least some symptomatic disease and epidemiological relevance, as often reinfections occur with negligible symptoms and

high Ct values, which are of less public health significance.<sup>15</sup> Of note that PCR testing in Qatar is done at a mass scale, where about 5% of the population are tested every week.<sup>16</sup> About 75% of those diagnosed are diagnosed not because of appearance of symptoms, but because of routine testing.<sup>16</sup>

Only the first PCR-positive test for a specific variant of interest was included for each case. A control was defined as the first PCR-negative test for any individual tested for clinical suspicion during the study period.<sup>16-20</sup> Prior infection was defined as a PCR-confirmed infection  $\geq 90$  days before a new PCR-positive test.<sup>4,21</sup> Individuals PCR-positive during the 90 days preceding the PCR test were therefore excluded from both cases and controls. These inclusion and exclusion criteria were implemented to minimize different types of potential bias, as informed by prior analyses.<sup>16</sup>

Each person who had a PCR-positive test result and hospital admission was subject to an infection severity assessment every three days until discharge or death, regardless of the length of the hospital stay or the time between the PCR-positive test and the final disease outcome. Classification of COVID-19 case severity (acute-care hospitalization),<sup>22</sup> criticality (intensive-care-unit (ICU) hospitalization),<sup>22</sup> and fatality<sup>23</sup> followed World Health Organization (WHO) guidelines, and assessments were made by trained medical personnel using individual chart reviews (Section S3).

The latter protocol for infection severity assessment was applied for Alpha, Beta, and Delta cases. However, with the recency of the Omicron epidemic wave, assessment of severity, criticality, and fatality of Omicron cases was completed for only a small number of cases. Therefore, only for Omicron cases, any acute-bed hospital admission associated with infection was used as a proxy for COVID-19 severity, and any ICU-bed hospital admission associated

with infection was used as a proxy for COVID-19 criticality. Alpha, Beta, Delta, and Omicron cases that progressed to severe,<sup>22</sup> critical,<sup>22</sup> or fatal<sup>23</sup> COVID-19 between the PCR-positive test result and the end of the study were classified based on their worst outcome, starting with death, followed by critical disease, and then severe disease.

Effectiveness of prior infection in preventing severe, critical, or fatal COVID-19 reinfection was also estimated, applying the same methodology. Here, cases (PCR-positive persons with a variant infection that progressed to a severe, critical, or fatal COVID-19) were exact matched to controls (PCR-negative persons) using the matching criteria specified above for each variant type.

The study was approved by the Hamad Medical Corporation and Weill Cornell Medicine-Qatar Institutional Review Boards with a waiver of informed consent. Reporting of the study followed STROBE guidelines (Table S5).

### **Statistical analysis**

All records of PCR testing in Qatar were examined for the selection of cases and controls and ascertainment of prior infection status. However, only matched samples of cases and controls were included in the analysis. Cases and controls were described using frequency distributions and measures of central tendency and compared using standardized mean differences (SMDs). SMD is defined as the difference in the mean of a covariate between groups divided by the pooled standard deviation, with  $SMD < 0.1$  indicating optimal balance across groups.

$PE_S$  was derived as one minus the ratio of the odds of prior infection in cases (PCR-positive persons with variant infection), to the odds of prior infection in controls (PCR-negative persons):<sup>3</sup>



$PE_S = 1 - \text{odds ratio of prior infection among cases versus controls}$  .

Odds ratios and associated 95% confidence intervals (CIs) were derived using conditional logistic regression, factoring the matching in the study design. This analytical approach minimizes potential bias that could arise due to variation in epidemic phase<sup>1,24</sup> or other confounders.<sup>6,11-14,25,26</sup> CIs were not adjusted for multiplicity. Interactions were not investigated.

Two types of sensitivity analyses were conducted to assess the robustness of estimates of  $PE_S$ . The first estimated  $PE_S$  by additionally adjusting for each of vaccination status and time from prior infection to PCR test in the conditional logistic regression. The second estimated  $PE_S$  after excluding all individuals with a record of vaccination prior to the PCR test used for defining cases and controls (Figures S3 and S4).

### **Caveats and limitations**

Individual-level data on co-morbid conditions were not available; therefore, they could not be explicitly factored into our analysis. However, only a small proportion of the study population may have had serious co-morbid conditions. Only 9% of the population of Qatar are  $\geq 50$  years of age,<sup>5,6</sup> and 60% are young, expatriate craft and manual workers working in mega-development projects.<sup>13,14,27</sup> The national list of persons prioritized to receive the vaccine during the first phase of vaccine roll-out included only 19,800 individuals of all age groups with serious co-morbid conditions. Matching of cases and controls on age may have indirectly and partially adjusted for presence of co-morbidities. With the young population of Qatar, our findings may not be generalizable to other countries where elderly citizens constitute a larger proportion of the total population. Of note that the prescription for matching included the epidemiologically relevant covariates as informed by two-year investigation of the local SARS-CoV-2 epidemiology in

Qatar.<sup>6,11-14</sup> The prescription was also recently shown to provide an adequate control of the differences in the risk of exposure to the infection.<sup>28</sup>

With the relatively young population of Qatar,<sup>6,29</sup> the lower severity of Omicron,<sup>30</sup> and the time lag between infection and severe forms of COVID-19, there were small number of confirmed severe, critical, and fatal COVID-19 cases to precisely estimate  $PE_S$  against COVID-19 hospitalization and death due to reinfection.

$PE_S$  may vary based on the variant status of the first (prior) infection, but our analysis did not factor the variant status of the prior infection. However, the consistency of our estimates of  $PE_S$  against Alpha and Beta in different analyses at different times (Table 1),<sup>3,31,32</sup> as well as the high  $PE_S$  against Alpha, Beta, Delta, and original virus (Table 1),<sup>3,4,21,31-33</sup> seem to suggest that the differences in  $PE_S$  based on the variant status of the prior infection may not be considerable, except perhaps for Omicron. It remains to be seen whether exposure to Omicron may entail inferior  $PE_S$  against future variants that are similar to Alpha, Beta, and Delta.

The study is based on PCR tests done on individuals currently in Qatar. Qatar has a diverse expatriate population, and it is possible that some persons may have had a prior infection diagnosis while traveling abroad to visit family or for vacation, but which would not have been captured in our national databases. However, this is not likely to affect our estimates. It has already been shown that even considerable levels of misclassification of prior infection status had a minimal impact on estimated  $PE_S$ ,<sup>3</sup> a key strength of the test-negative design.<sup>3</sup>

$PE_S$  was assessed using an observational, test-negative, case-control study design,<sup>3</sup> rather than a cohort study design where individuals are followed up over time. However, the cohort study design applied in earlier analyses to estimate  $PE_S$  in the same population of Qatar yielded findings similar to those of the test-negative case-control design,<sup>3,4,31-33</sup> supporting the validity of

this design in estimating  $PEs$ . It even appears that the test-negative study design may be less susceptible to some forms of bias than the cohort study design.<sup>3</sup>

Nonetheless, one cannot exclude the possibility that in real-world data, bias could arise in unexpected ways, or from unknown sources, such as subtle differences in test-seeking behavior or changes in the pattern of testing with introduction of other testing modalities, such as rapid antigen testing.

Notwithstanding these limitations, consistent findings were reached in both the main and sensitivity analyses. Estimates for the effectiveness of prior infection against reinfection with the Alpha and Beta variants were also consistent and similar to those generated earlier in the same population of Qatar using cohort study designs.<sup>31,32</sup>

## **Section S2. Laboratory methods**

### **Real-time reverse-transcription polymerase chain reaction testing**

Nasopharyngeal and/or oropharyngeal swabs were collected for polymerase chain reaction (PCR) testing and placed in Universal Transport Medium (UTM). Aliquots of UTM were: extracted on a QIAasymphony platform (QIAGEN, USA) and tested with real-time reverse-transcription PCR (RT-qPCR) using TaqPath COVID-19 Combo Kits (Thermo Fisher Scientific, USA) on an ABI 7500 FAST (Thermo Fisher, USA); tested directly on the Cepheid GeneXpert system using the Xpert Xpress SARS-CoV-2 (Cepheid, USA); or loaded directly into a Roche cobas 6800 system and assayed with a cobas SARS-CoV-2 Test (Roche, Switzerland). The first assay targets the viral S, N, and ORF1ab gene regions. The second targets the viral N and E-gene regions, and the third targets the ORF1ab and E-gene regions.

All PCR testing was conducted at the Hamad Medical Corporation Central Laboratory or Sidra Medicine Laboratory, following standardized protocols. As described above, all PCR testing was performed with extensively used, investigated, and validated commercial platforms having essentially 100% sensitivity and specificity. There is also no evidence that the sensitivity and specificity of these diagnostic methods are affected by the nature of the Omicron variant.<sup>7</sup>

### **Classification of infections by variant type**

Surveillance for SARS-CoV-2 variants in Qatar is mainly based on viral genome sequencing and multiplex RT-qPCR variant screening<sup>34</sup> of random positive clinical samples,<sup>8,9,16-18,35</sup> complemented by deep sequencing of wastewater samples.<sup>8,36</sup>

Between March 23, 2021 and November 18, 2021 (prior to suspected introduction of the Omicron variant), RT-qPCR genotyping of 19,234 randomly collected SARS-CoV-2-positive

specimens on a weekly basis identified 3,494 (18.2%) Alpha (B.1.1.7)-like cases, 5,768 (30.0%) Beta (B.1.351)-like cases, 9,914 (51.5%) “other” variant cases, and 58 (0.3%) B.1.375-like or B.1.258-like cases.<sup>8,9</sup> Since these samples were chosen randomly on a weekly basis from all samples of cases diagnosed during that week, it is not likely that these samples are biased relative to all diagnosed cases to affect the estimated  $PE_S$ .

The accuracy of the RT-qPCR genotyping was verified against either Sanger sequencing of the receptor-binding domain (RBD) of SARS-CoV-2 surface glycoprotein (S) gene, or by viral whole-genome sequencing on a Nanopore GridION sequencing device. From 236 random samples (27 Alpha-like, 186 Beta-like, and 23 “other” variants), PCR genotyping results for Alpha-like, Beta-like, and ‘other’ variants were in 88.8% (23 out of 27), 99.5% (185 out of 186), and 100% (23 out of 23) agreement with the SARS-CoV-2 lineages assigned by sequencing.

Within the “other” variant category, Sanger sequencing and/or Illumina sequencing of the RBD of SARS-CoV-2 spike gene on 728 random samples confirmed that 701 (96.3%) were Delta cases and 17 (2.3%) were other variant cases, with 10 (1.4%) samples failing lineage assignment.<sup>6,8</sup> Accordingly, a Delta case was proxied as any “other” case identified through the RT-qPCR based variant screening.

All the variant RT-qPCR screening was conducted at the Sidra Medicine Laboratory following standardized protocols.

Surveillance for Omicron infection was performed using the TaqPath COVID-19 Combo Kit platform (Thermo Fisher Scientific, USA<sup>10</sup>) applying the criterion of an RT-qPCR Ct value  $\leq 30$  for both the N and ORF1ab genes, but a negative outcome for the S gene (S-gene “target failure”).

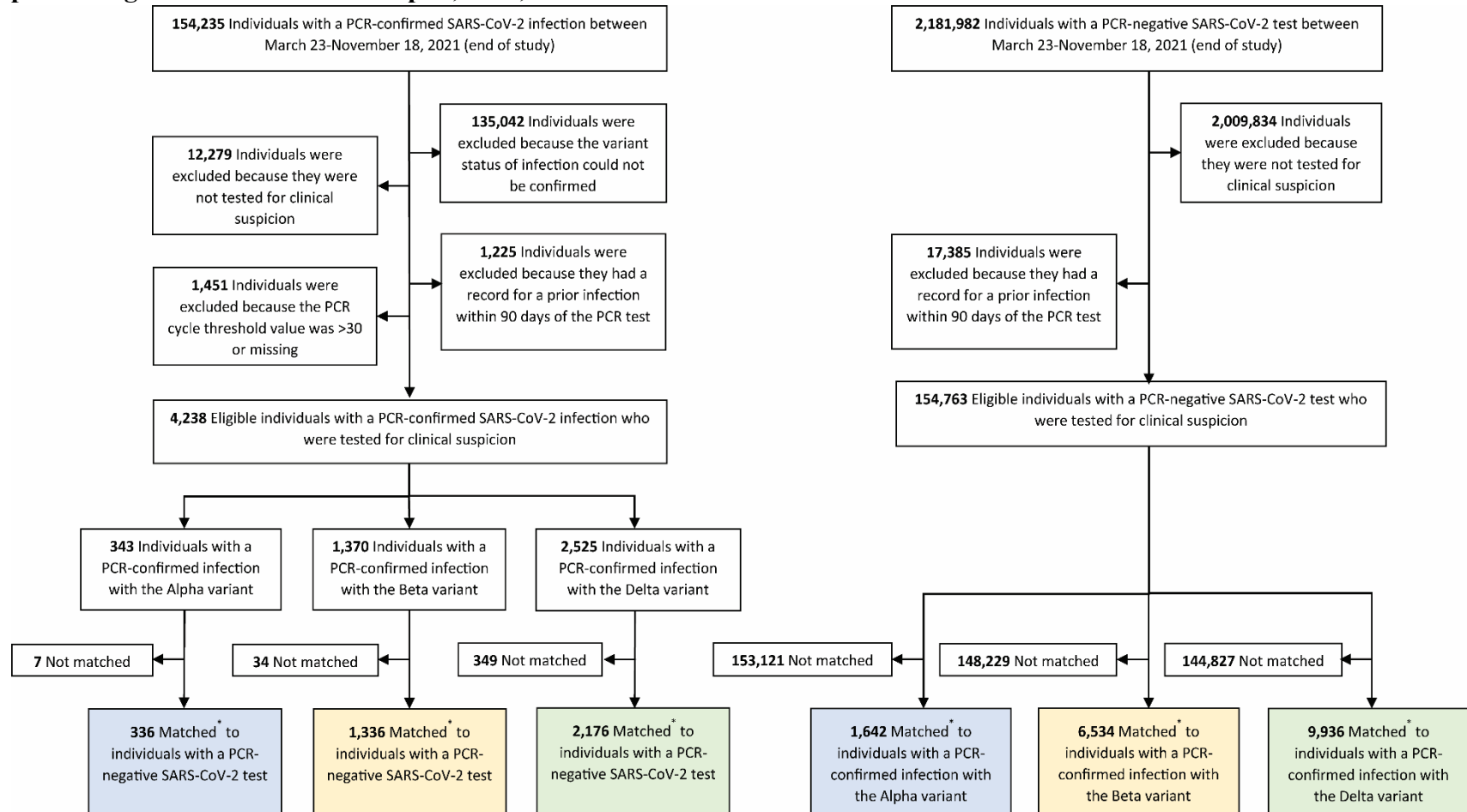
### **Section S3. COVID-19 severity, criticality, and fatality classification**

Severe Coronavirus Disease 2019 (COVID-19) disease was defined per the World Health Organization (WHO) classification as a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected person with “oxygen saturation of  $<90\%$  on room air, and/or respiratory rate of  $>30$  breaths/minute in adults and children  $>5$  years old (or  $\geq 60$  breaths/minute in children  $<2$  months old or  $\geq 50$  breaths/minute in children 2-11 months old or  $\geq 40$  breaths/minute in children 1–5 years old), and/or signs of severe respiratory distress (accessory muscle use and inability to complete full sentences, and, in children, very severe chest wall indrawing, grunting, central cyanosis, or presence of any other general danger signs)”.<sup>22</sup> Detailed WHO criteria for classifying SARS-CoV-2 infection severity can be found in the WHO technical report.<sup>22</sup>

Critical COVID-19 disease was defined per WHO classification as a SARS-CoV-2 infected person with “acute respiratory distress syndrome, sepsis, septic shock, or other conditions that would normally require the provision of life sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy”.<sup>22</sup> Detailed WHO criteria for classifying SARS-CoV-2 infection criticality can be found in the WHO technical report.<sup>22</sup>

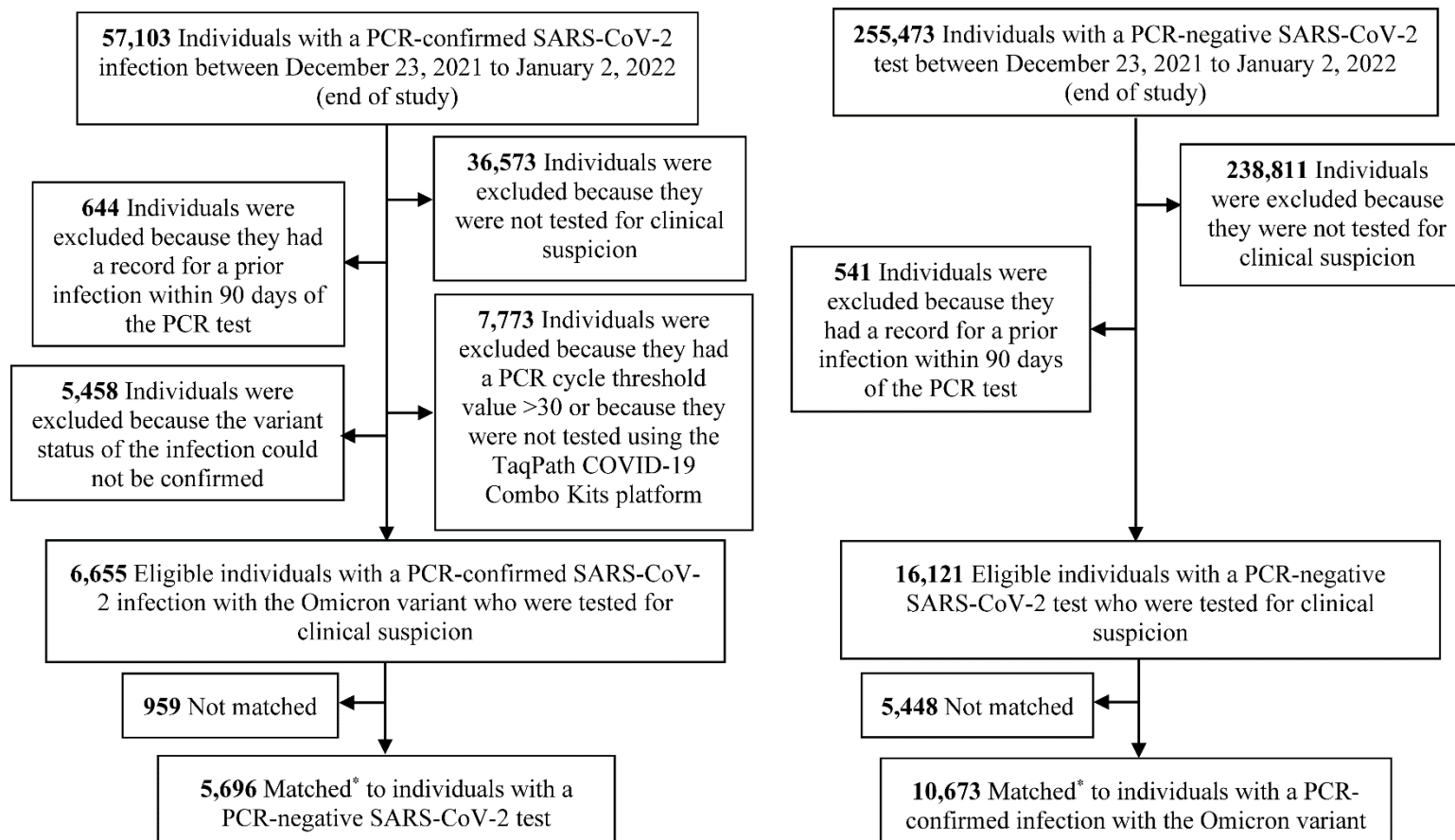
COVID-19 death was defined per WHO classification as “a death resulting from a clinically compatible illness, in a probable or confirmed COVID-19 case, unless there is a clear alternative cause of death that cannot be related to COVID-19 disease (e.g. trauma). There should be no period of complete recovery from COVID-19 between illness and death. A death due to COVID-19 may not be attributed to another disease (e.g. cancer) and should be counted independently of preexisting conditions that are suspected of triggering a severe course of COVID-19”. Detailed WHO criteria for classifying COVID-19 death can be found in the WHO technical report.<sup>23</sup>

**Figure S1. Flowchart describing the population selection process for investigating the effectiveness of prior infection in preventing reinfection with the Alpha, Beta, and Delta variants.**



\* Individuals with a PCR-confirmed infection with SARS-CoV-2 Alpha, Beta or Delta variant were exact matched in a 1:5 ratio by sex, 10-year age group, nationality, and PCR test week to the first eligible individual with a PCR-negative SARS-CoV-2 test. Prior infection records were retrieved for all matched individuals.

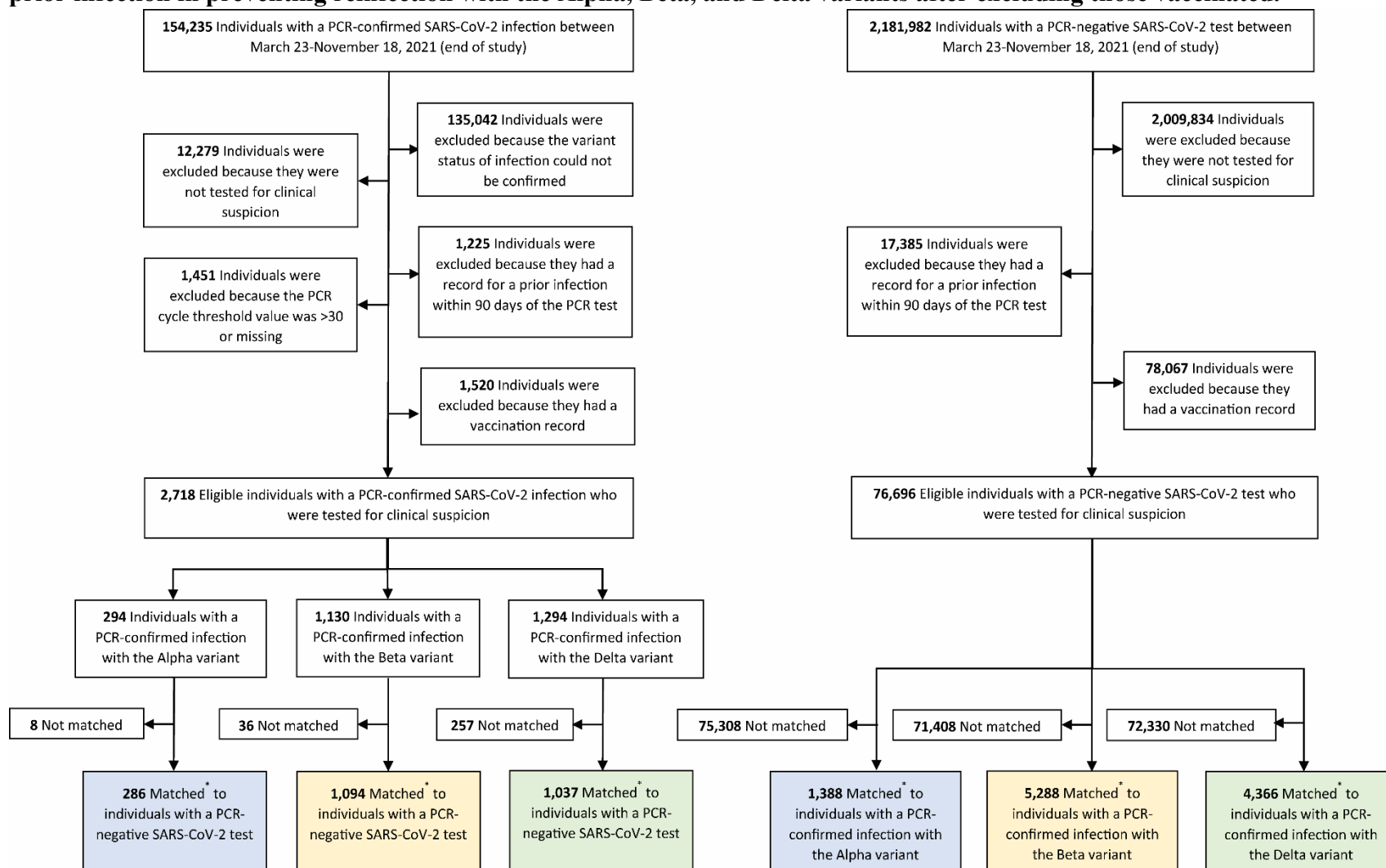
**Figure S2. Flowchart describing the population selection process for investigating the effectiveness of prior infection in preventing reinfection with the Omicron variant.**



\*Individuals with a PCR-confirmed infection with SARS-CoV-2 Omicron variant were exact matched in a 1:3 ratio by sex, 10-year age group, nationality, and PCR test date to the first eligible individual with a PCR-negative SARS-CoV-2 test. Prior infection records were retrieved for all matched individuals.

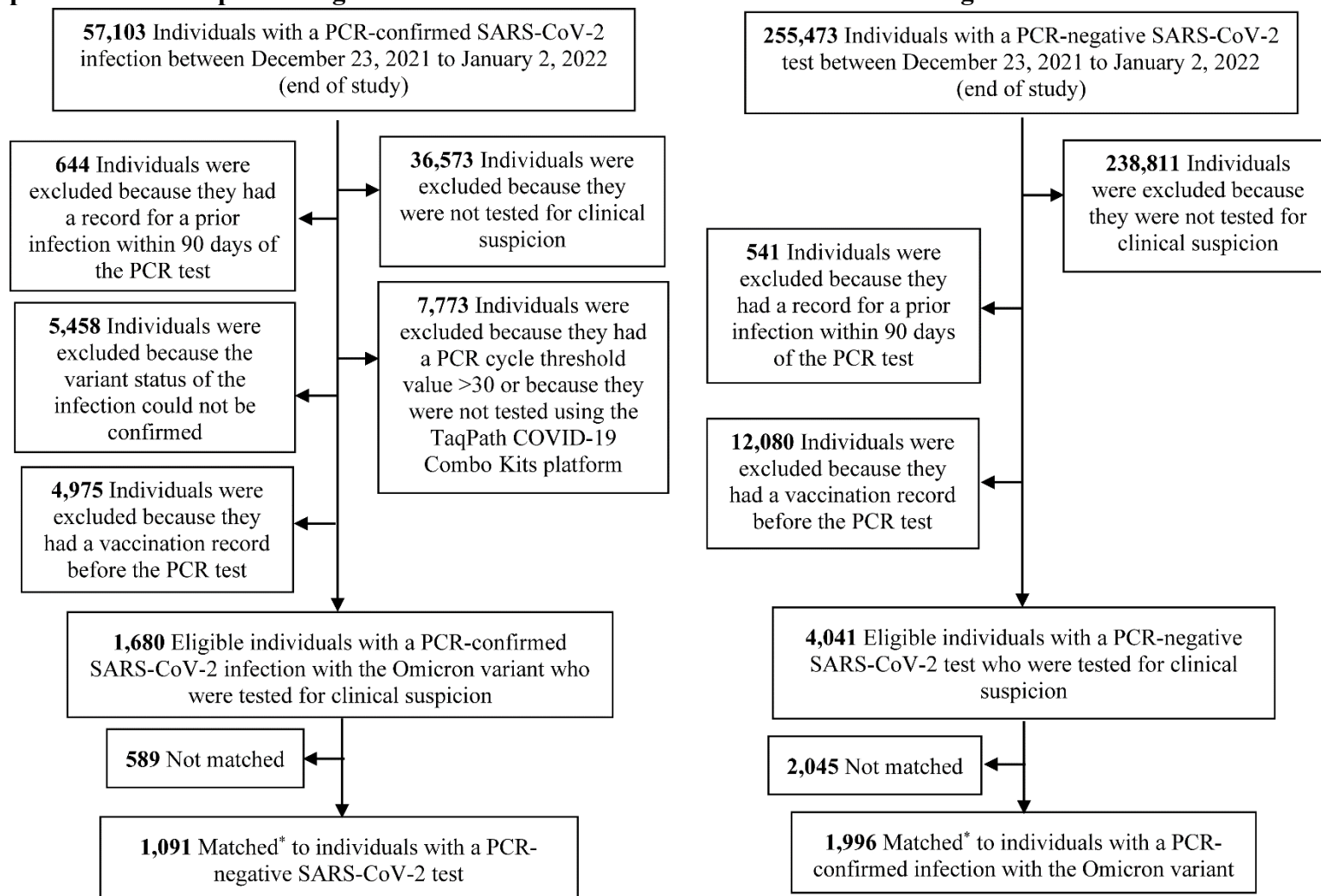


**Figure S3. Flowchart describing the population selection process for the sensitivity analysis investigating the effectiveness of prior infection in preventing reinfection with the Alpha, Beta, and Delta variants after excluding those vaccinated.**



\*Individuals with a PCR-confirmed infection with SARS-CoV-2 Alpha, Beta or Delta variant were exact matched in a 1:5 ratio by sex, 10-year age group, nationality, and PCR test week to the first eligible individual with a PCR-negative SARS-CoV-2 test. Prior infection records were retrieved for all matched individuals.

**Figure S4. Flowchart describing the population selection process for the sensitivity analysis investigating the effectiveness of prior infection in preventing reinfection with the Omicron variant after excluding those vaccinated.**



\*Individuals with a PCR-confirmed infection with SARS-CoV-2 Omicron variant were exact matched in a 1:3 ratio by sex, 10-year age group, nationality, and PCR test date to the first eligible individual with a PCR-negative SARS-CoV-2 test. Prior infection records were retrieved for all matched individuals.

**Table S1. Characteristics of matched cases (PCR-positive persons with Alpha, Beta, or Delta infections, respectively) and controls (PCR-negative persons).**

Characteristics	Cases* (PCR-confirmed infection with Alpha)	Controls* (PCR-negative)	SMD†	Cases* (PCR-confirmed infection with Beta)	Controls* (PCR-negative)	SMD†	Cases* (PCR-confirmed infection with Delta)	Controls* (PCR-negative)	SMD†
	N=336	N=1,642		N=1,336	N=6,534		N=2,176	N=9,936	
<b>Median age (IQR) — years</b>	31 (23-39)	31 (23-39)	0.00‡	35 (27-42)	34 (27-42)	0.01‡	31 (20-40)	31 (19-40)	0.04‡
<b>Age group — no. (%)§</b>									
<20 years	70 (20.8)	333 (20.3)		157 (11.8)	745 (11.4)		538 (24.7)	2,484 (25.0)	
20-29 years	75 (22.3)	372 (22.7)		272 (20.4)	1,346 (20.6)		436 (20.0)	2,056 (20.7)	
30-39 years	113 (33.6)	560 (34.1)		471 (35.3)	2,323 (35.6)		615 (28.3)	2,904 (29.2)	
40-49 years	56 (16.7)	267 (16.3)	0.02	311 (23.3)	1,522 (23.3)	0.04	373 (17.1)	1,626 (16.4)	0.05
50-59 years	18 (5.4)	90 (5.5)		97 (7.3)	477 (7.3)		153 (7.0)	635 (6.4)	
60-69 years	3 (0.9)	15 (0.9)		24 (1.8)	112 (1.7)		47 (2.2)	174 (1.8)	
70+ years	1 (0.3)	5 (0.3)		4 (0.3)	9 (0.1)		14 (0.6)	53 (0.5)	
<b>Sex</b>									
Male	178 (53.0)	861 (52.4)	0.01	937 (70.1)	4,626 (70.8)	0.01	1,108 (50.9)	5,077 (51.1)	0.00
Female	158 (47.0)	781 (47.6)		399 (29.9)	1,908 (29.2)		1,068 (49.1)	4,859 (48.9)	
<b>Nationality¶</b>									
Bangladeshi	21 (6.3)	105 (6.4)		117 (8.8)	581 (8.9)		129 (5.9)	621 (6.3)	
Egyptian	19 (5.7)	95 (5.8)		71 (5.3)	351 (5.4)		160 (7.4)	718 (7.2)	
Filipino	43 (12.8)	215 (13.1)		132 (9.9)	655 (10.0)		175 (8.0)	866 (8.7)	
Indian	55 (16.4)	275 (16.7)		309 (23.1)	1,545 (23.6)		202 (9.3)	1,004 (10.1)	
Nepalese	17 (5.1)	85 (5.2)	0.04	163 (12.2)	806 (12.3)	0.04	38 (1.7)	184 (1.9)	0.12
Pakistani	13 (3.9)	63 (3.8)		55 (4.1)	269 (4.1)		67 (3.1)	324 (3.3)	
Qatari	89 (26.5)	445 (27.1)		186 (13.9)	930 (14.2)		846 (38.9)	4,127 (41.5)	
Sri Lankan	16 (4.8)	78 (4.8)		62 (4.6)	301 (4.6)		28 (1.3)	130 (1.3)	
Sudanese	8 (2.4)	36 (2.2)		40 (3.0)	197 (3.0)		68 (3.1)	280 (2.8)	
Other nationalities**	55 (16.4)	245 (14.9)		201 (15.0)	899 (13.8)		463 (21.3)	1,682 (16.9)	
<b>PCR test calendar month**</b>									
March	51 (15.2)	349 (21.3)		213 (15.9)	1,375 (21.0)		2 (0.1)	10 (0.1)	
April	227 (67.6)	1,018 (62.0)		970 (72.6)	4,417 (67.6)		14 (0.6)	67 (0.7)	
May	29 (8.6)	130 (7.9)		102 (7.6)	497 (7.6)		104 (4.8)	495 (5.0)	
June	11 (3.3)	50 (3.0)		3 (0.2)	23 (0.4)		82 (3.8)	409 (4.1)	
July	12 (3.6)	78 (4.8)	0.18	24 (1.8)	106 (1.6)	0.14	538 (24.7)	2,384 (24.0)	0.03
August	6 (1.8)	17 (1.0)		24 (1.8)	116 (1.8)		800 (36.8)	3,704 (37.3)	
September	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		370 (17.0)	1,678 (16.9)	
October	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		217 (10.0)	977 (9.8)	
November	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		49 (2.3)	212 (2.1)	

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction; SMD, standardized mean difference.

\*Cases and controls were exact matched one-to-five by sex, 10-year age group, nationality, and calendar week of PCR test.

†SMD is the difference in the mean of a covariate between groups divided by the pooled standard deviation. An SMD<0.1 indicates optimal balance in matching.

‡SMD is for the mean difference between groups divided by the pooled standard deviation.

§The average age within each 10-year age stratum for cases and controls was similar within ±1 year except for the small stratum of 70+ years.

¶Nationalities were chosen to represent the most populous groups in Qatar.

\*\*These comprise 20 other nationalities in Qatar in the Alpha variant analysis, 29 other nationalities in the Beta variant analysis, and 30 other nationalities in the Delta variant analysis.

\*\*Cases and controls were exact matched using calendar week of PCR test, but we opted to report the distribution by calendar month for brevity. Accordingly, some cases and controls who were tested in the same week may appear in different calendar months.

**Table S2. Characteristics of matched cases (PCR-positive persons with Omicron infection) and controls (PCR-negative persons).**

Characteristics	Cases*	Controls*	SMD†
	(PCR-confirmed infection with Omicron)	(PCR-negative)	
	<b>N=5,696</b>	<b>N=10,673</b>	
<b>Median age (IQR) — years</b>	33 (25-40)	32 (24-40)	0.04‡
<b>Age group — no. (%)§</b>			
<20 years	923 (16.2)	1,876 (17.6)	
20-29 years	1,187 (20.8)	2,339 (21.9)	
30-39 years	2,078 (36.5)	3,721 (34.9)	
40-49 years	975 (17.1)	1,718 (16.1)	0.06
50-59 years	369 (6.5)	707 (6.6)	
60-69 years	118 (2.1)	232 (2.2)	
70+ years	46 (0.8)	80 (0.8)	
<b>Sex</b>			
Male	3,148 (55.3)	5,877 (55.1)	
Female	2,548 (44.7)	4,796 (44.9)	0.00
<b>Nationality¶</b>			
Bangladeshi	157 (2.8)	323 (3.0)	
Egyptian	476 (8.4)	746 (7.0)	
Filipino	1,003 (17.6)	1,569 (14.7)	
Indian	1,027 (18.0)	1,880 (17.6)	
Nepalese	170 (3.0)	219 (2.1)	
Pakistani	160 (2.8)	307 (2.9)	0.18
Qatari	1,276 (22.4)	3,126 (29.3)	
Sri Lankan	86 (1.5)	122 (1.1)	
Sudanese	274 (4.8)	550 (5.2)	
Other nationalities**	1,067 (18.7)	1,831 (17.2)	
<b>PCR test date</b>			
23 December, 2021	244 (4.3)	575 (5.4)	
24 December, 2021	127 (2.2)	309 (2.9)	
25 December, 2021	287 (5.0)	605 (5.7)	
26 December, 2021	540 (9.5)	1,123 (10.5)	
27 December, 2021	657 (11.5)	1,377 (12.9)	
28 December, 2021	887 (15.6)	1,585 (14.9)	0.13
29 December, 2021	1,043 (18.3)	1,807 (16.9)	
30 December, 2021	1,199 (21.1)	1,879 (17.6)	
31 December, 2021	670 (11.8)	1,292 (12.1)	
01 January, 2022	42 (0.7)	121 (1.1)	

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction; SMD, standardized mean difference.

†Cases and controls were matched one-to-three by sex, 10-year age group, nationality, and PCR test date.

‡SMD is the difference in the mean of a covariate between groups divided by the pooled standard deviation. An SMD<0.1 indicates adequate matching.

§SMD is for the mean difference between groups divided by the pooled standard deviation.

¶The average age within each 10-year age stratum for cases and controls was similar within ±1.

\*\*Nationalities were chosen to represent the most populous groups in Qatar.

\*\*These comprise 44 other nationalities in Qatar.

**Table S3. Representativeness of study participants.**

Category	
Disease, problem, or condition under investigation	Effectiveness of prior infection in preventing reinfection with SARS-CoV-2 Alpha, Beta, Delta, and Omicron variants
Special considerations related to	
Sex and gender	The effectiveness estimates were derived by comparing cases (PCR-positive for Alpha, Beta, Delta, or Omicron) and controls (PCR-negative) with respect to prior infection. Cases and controls were exact-matched by sex to control for potential differences in the risk of exposure to SARS-CoV-2 infection by sex.
Age	Cases and controls were exact-matched by 10-year age group to control for potential differences in the risk of exposure to SARS-CoV-2 infection by age. Nonetheless, with the young population of Qatar, our findings may not be generalizable to other countries where elderly citizens constitute a larger proportion of the total population.
Race or ethnicity group	Cases and controls were exact-matched by nationality to control for potential differences in the risk of exposure to SARS-CoV-2 infection by nationality. Nationality is associated with race and ethnicity in the population of Qatar.
Geography	Cases and controls were exact-matched by nationality to control for potential differences in the risk of exposure to SARS-CoV-2 infection by nationality. Qatar has unusually diverse demographics in that 89% of the population are international expatriate residents coming from over 150 countries from all world regions.
Other considerations	Individual-level data on co-morbid conditions were not available, but only a small proportion of the study population may have had serious co-morbid conditions. Only 9% of the population of Qatar are $\geq 50$ years of age (older age as proxy for co-morbidities). The national list of persons prioritized to receive the vaccine during the first phase of vaccine roll-out included only 19,800 individuals of all age groups with serious co-morbid conditions.
Overall representativeness of this study	The study samples were broadly representative of the diverse by national background, but young and predominantly male total population of Qatar. While there could be differences in the risk of exposure to SARS-CoV-2 infection by sex, age, and nationality, cases and controls were exact-matched by these factors to control for their potential impact on our estimates for effectiveness of prior infection. Given that only 9% of the population of Qatar are $\geq 50$ years of age and the limited proportion of the population with significant co-morbidities, our estimates of effectiveness may not be generalizable to other countries where elderly citizens constitute a larger proportion of the total population or where co-morbid conditions are prevalent.

**Table S4. Effectiveness of SARS-CoV-2 prior infection against reinfection with Alpha, Beta, Delta, or Omicron variant, adjusting for time between prior infection and PCR test.**

	Cases* (PCR-positive)		Controls* (PCR-negative)		Effectiveness in % (95% CI) <sup>†</sup>
	Prior infection	No prior infection	Prior infection	No prior infection	
<b>Alpha</b>					
3-8 months	1	334	43	1,548	89.4 (22.6 to 98.5)
9-14 months	1	334	51	1,548	91.0 (34.5 to 98.8)
≥15 months	--	--	--	--	--
<b>Beta</b>					
3-8 months	3	1,322	186	6,084	92.6 (76.7 to 97.6)
9-14 months	11	1,322	264	6,084	81.2 (65.5 to 89.8)
≥15 months	--	--	--	--	--
<b>Delta</b>					
3-8 months	10	2,153	602	8,782	93.4 (87.6 to 96.5)
9-14 months	10	2,153	454	8,782	91.1 (83.3 to 95.3)
≥15 months	3	2,153	98	8,782	87.1 (59.4 to 95.9)
<b>Omicron</b>					
3-8 months	94	5,284	460	9,053	64.0 (54.7-71.4)
9-14 months	191	5,284	630	9,053	47.2 (37.5-55.4)
≥15 months	127	5,284	530	9,053	59.6 (50.7-67.0)

\*Cases and controls were exact matched one-to-five by sex, 10-year age group, nationality, and calendar week of PCR test in the Alpha, Beta, and Delta analyses (March 23-November 18, 2021; Figure S1), and one-to-three by sex, 10-year age group, nationality, and PCR test date in the Omicron analysis (December 23, 2021- Jan 2, 2022; Figure S2).

<sup>†</sup>Effectiveness of prior infection in preventing reinfection was estimated using the test-negative, case-control study design.<sup>3</sup>

**Table S5. STROBE checklist for case-control studies.**

	<b>Item No</b>	<b>Recommendation</b>	<b>Main text page</b>
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Letter main text p.3
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	NA
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Letter main text p.3
Objectives	3	State specific objectives, including any prespecified hypotheses	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design')
<b>Methods</b>			
Study design	4	Present key elements of study design	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design')
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Supp. Section S1 ('Study population, data sources, and study design') & Supp. Figures S1-S2
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls (b) For matched studies, give matching criteria and the number of controls per case	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design') & Supp. Figures S1-S2
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design' & 'Statistical analysis'), Supp. Sections S2 & S3
Data sources/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design' & 'Statistical analysis') & Supp. Tables S1-S2
Bias	9	Describe any efforts to address potential sources of bias	Letter main text p.3, Table 1, Supp. Section S1 ('Study population, data sources, and study design' & 'Statistical analysis'), & Supp. Figures S3-S4 & Supp. Table S4
Study size	10	Explain how the study size was arrived at	Supp. Section S1 ('Study population, data sources, and study design') & Supp. Figures S1-S2
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Supp. Section S1 ('Study population, data sources, and study design' & 'Statistical analysis') & Supp. Tables S1-S2
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Supp. Section S1 ('Statistical analysis') & Table 1
		(b) Describe any methods used to examine subgroups and interactions	Supp. Section S1 ('Statistical analysis'), Supp. Figures S3-S4, Table 1, & Supp. Table S4
	(c) Explain how missing data were addressed	NA, see Supp. Section S1 ('Study population, data sources, and study design')	
	(d) If applicable, explain how matching of cases and controls was addressed	Letter main text p.3 & Supp. Section S1 ('Study population, data sources, and study design') & Supp. Tables S1-S2	
	(e) Describe any sensitivity analyses	Supp. Section S1 ('Statistical analysis') Supp. Figures S3-S4, Table 1, & Supp. Table S4	
<b>Results</b>			

Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Supp. Figures S1-S2
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	Supp. Tables S1-S3 NA, see Supp. Section S1 ('Study population, data sources, and study design')
Outcome data	15	Report numbers in each exposure category, or summary measures of exposure	Letter main text p.4 & Table 1
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Letter main text p.4 & Table 1 Supp. Tables S1-S2 NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Letter main text p.4, Table 1 & Supp. Table S4
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Letter main text p.4
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Letter main text p.4 & Supp. Section S1 ('Caveats and limitations')
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Letter main text p.4
Generalisability	21	Discuss the generalisability (external validity) of the study results	Supp. Section S1 ('Caveats and limitations') & Supp. Table S3
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Letter main text p.7

Abbreviations: NA, not applicable; p. page; Supp. Supplementary Appendix.



## References

1. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. *Vaccine* 2013;31:2165-8.
2. Verani JR, Baqui AH, Broome CV, et al. Case-control vaccine effectiveness studies: Preparation, design, and enrollment of cases and controls. *Vaccine* 2017;35:3295-302.
3. Ayoub HH, Tomy M, Chemaitelly H, et al. Estimating protection afforded by prior infection in preventing reinfection: Applying the test-negative study design. *medRxiv* 2022:2022.01.02.22268622.
4. Abu-Raddad LJ, Chemaitelly H, Coyle P, et al. SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy. *EClinicalMedicine* 2021;35:100861.
5. Planning and Statistics Authority-State of Qatar. Qatar Monthly Statistics. Available from: <https://www.psa.gov.qa/en/pages/default.aspx>. Accessed on: May 26, 2020. 2020.
6. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Characterizing the Qatar advanced-phase SARS-CoV-2 epidemic. *Sci Rep* 2021;11:6233.
7. World Health Organization. Tracking SARS-CoV-2 variants. Available from: <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>. 2021.
8. Qatar viral genome sequencing data. Data on randomly collected samples. <https://www.gisaid.org/phylogenetics/global/nextstrain/>. 2021. at <https://www.gisaid.org/phylogenetics/global/nextstrain/>.
9. Hasan MR, Kalikiri MKR, Mirza F, et al. Real-Time SARS-CoV-2 Genotyping by High-Throughput Multiplex PCR Reveals the Epidemiology of the Variants of Concern in Qatar. *Int J Infect Dis* 2021;112:52-4.
10. Thermo Fisher Scientific. TaqPath™ COVID-19 CE-IVD RT-PCR Kit instructions for use. Available from: [https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019215\\_TaqPathCOVID-19\\_CE-IVD\\_RT-PCR%20Kit\\_IFU.pdf](https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019215_TaqPathCOVID-19_CE-IVD_RT-PCR%20Kit_IFU.pdf). Accessed on December 02, 2020. 2020.
11. Ayoub HH, Chemaitelly H, Seedat S, et al. Mathematical modeling of the SARS-CoV-2 epidemic in Qatar and its impact on the national response to COVID-19. *J Glob Health* 2021;11:05005.
12. Coyle PV, Chemaitelly H, Ben Hadj Kacem MA, et al. SARS-CoV-2 seroprevalence in the urban population of Qatar: An analysis of antibody testing on a sample of 112,941 individuals. *iScience* 2021;24:102646.
13. Al-Thani MH, Farag E, Bertollini R, et al. SARS-CoV-2 Infection Is at Herd Immunity in the Majority Segment of the Population of Qatar. *Open Forum Infect Dis* 2021;8:ofab221.
14. Jeremijenko A, Chemaitelly H, Ayoub HH, et al. Herd Immunity against Severe Acute Respiratory Syndrome Coronavirus 2 Infection in 10 Communities, Qatar. *Emerg Infect Dis* 2021;27:1343-52.
15. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Effect of vaccination and of prior infection on infectiousness of vaccine breakthrough infections and reinfections. *medRxiv* In press at *Nature Communications* 2021:2021.07.28.21261086.
16. Chemaitelly H, Tang P, Hasan MR, et al. Waning of BNT162b2 Vaccine Protection against SARS-CoV-2 Infection in Qatar. *N Engl J Med* 2021;385:e83.
17. Abu-Raddad LJ, Chemaitelly H, Butt AA, National Study Group for Covid Vaccination. Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. *N Engl J Med* 2021;385:187-9.
18. Chemaitelly H, Yassine HM, Benslimane FM, et al. mRNA-1273 COVID-19 vaccine effectiveness against the B.1.1.7 and B.1.351 variants and severe COVID-19 disease in Qatar. *Nat Med* 2021;27:1614-21.
19. Tang P, Hasan MR, Chemaitelly H, et al. BNT162b2 and mRNA-1273 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. *Nat Med* 2021;27:2136-43.

20. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Waning of mRNA-1273 vaccine effectiveness against SARS-CoV-2 infection in Qatar. medRxiv In press at the New England Journal of Medicine 2021:2021.12.16.21267902.
21. Kojima N, Shrestha NK, Klausner JD. A Systematic Review of the Protective Effect of Prior SARS-CoV-2 Infection on Repeat Infection. *Eval Health Prof* 2021;44:327-32.
22. World Health Organization. COVID-19 clinical management: living guidance. Available from: <https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1>. Accessed on: May 15 2021. 2021.
23. World Health Organization. International guidelines for certification and classification (coding) of COVID-19 as cause of death. Available from: [https://www.who.int/classifications/icd/Guidelines\\_Cause\\_of\\_Death\\_COVID-19-20200420-EN.pdf?ua=1](https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19-20200420-EN.pdf?ua=1). Document Number: WHO/HQ/DDI/DNA/CAT. Accessed on May 31, 2021. 2021.
24. Jacoby P, Kelly H. Is it necessary to adjust for calendar time in a test negative design?: Responding to: Jackson ML, Nelson JC. The test negative design for estimating influenza vaccine effectiveness. *Vaccine* 2013;31(April (17)):2165-8. *Vaccine* 2014;32:2942.
25. Pearce N. Analysis of matched case-control studies. *BMJ* 2016;352:i969.
26. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.
27. Planning and Statistics Authority- State of Qatar. Labor force sample survey. Available from: [https://www.psa.gov.qa/en/statistics/Statistical%20Releases/Social/LaborForce/2017/statistical\\_analyses\\_labor\\_force\\_2017\\_En.pdf](https://www.psa.gov.qa/en/statistics/Statistical%20Releases/Social/LaborForce/2017/statistical_analyses_labor_force_2017_En.pdf). Accessed on: May 01, 2020. 2017.
28. Abu-Raddad LJ, Chemaitelly H, Bertollini R, National Study Group for Covid Vaccination. Effectiveness of mRNA-1273 and BNT162b2 Vaccines in Qatar. *N Engl J Med* 2022.
29. Seedat S, Chemaitelly H, Ayoub HH, et al. SARS-CoV-2 infection hospitalization, severity, criticality, and fatality rates in Qatar. *Sci Rep* 2021;11:18182.
30. Wolter N, Jassat W, Walaza S, et al. Early assessment of the clinical severity of the SARS-CoV-2 Omicron variant in South Africa. medRxiv 2021:2021.12.21.21268116.
31. Chemaitelly H, Bertollini R, Abu-Raddad LJ, National Study Group for Covid Epidemiology. Efficacy of Natural Immunity against SARS-CoV-2 Reinfection with the Beta Variant. *N Engl J Med* 2021.
32. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Introduction and expansion of the SARS-CoV-2 B.1.1.7 variant and reinfections in Qatar: A nationally representative cohort study. *PLoS Med* 2021;18:e1003879.
33. Abu-Raddad LJ, Chemaitelly H, Malek JA, et al. Assessment of the Risk of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reinfection in an Intense Reexposure Setting. *Clin Infect Dis* 2021;73:e1830-e40.
34. Multiplexed RT-qPCR to screen for SARS-COV-2 B.1.1.7, B.1.351, and P.1 variants of concern V.3. dx.doi.org/10.17504/protocols.io.br9vm966. 2021. (Accessed June 6, 2021, at <https://www.protocols.io/view/multiplexed-rt-qpcr-to-screen-for-sars-cov-2-b-1-1-br9vm966>.)
35. Benslimane FM, Al Khatib HA, Al-Jamal O, et al. One Year of SARS-CoV-2: Genomic Characterization of COVID-19 Outbreak in Qatar. *Front Cell Infect Microbiol* 2021;11:768883.
36. Saththasivam J, El-Malah SS, Gomez TA, et al. COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar. *Sci Total Environ* 2021;774:145608.