


RESEARCH ARTICLE

Follow-up of SARS-CoV-2 positive subgroup from the Asymptomatic novel CORonavirus iNFection study

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Abstract

A nested longitudinal study within the Asymptomatic novel CORonavirus iNFection study followed participants with positive nasopharyngeal swab to query for development of symptoms and assess duration of positive reverse transcription-polymerase chain reaction (RT-PCR) test results. Of the 91 participants initially testing positive, 86 participated in follow-up approximately 14 days after study enrollment; of those 86 participants, 19 (22.1%) developed at least one symptom at any time after the initial positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) test result. The median number of days to symptom development after their initial positive test result was 6 (range 1–29 days). No participants reported a SARS-CoV-2-related hospitalization. The most frequently reported symptoms were fatigue or muscle aches (10.5%), headache (9.3%), fever (5.8%), and shortness of breath (5.8%). Of the 78 participants who submitted a nasopharyngeal swab for repeat RT-PCR testing, 17 (21.8%) remained positive at Day 14, 4 of which continued to test positive at Day 28. These findings reinforce the probable role of silent SARS-CoV-2 infections in community transmission, and that reliance on symptom development will miss a large proportion of infections. Broad testing programs not limited to individuals presenting with symptoms are critical for identifying persons with SARS-CoV-2 infection and ultimately slowing transmission.

KEYWORDS

asymptomatic infections, SARS-CoV-2, viral load

1 | INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in December 2019 and has since developed into a global pandemic.¹ As of January 11, 2021, over 22.1 million people have confirmed SARS-CoV-2 infection in the United States with over 371,000 deaths.² Assessing the number of persons affected across the disease spectrum and evaluating the clinical course of people testing positive for

SARS-CoV-2 yet presenting with no symptoms of the clinical syndrome caused by SARS-CoV-2 are important for understanding the nature of the pandemic and for slowing its spread.

A high proportion of asymptomatic cases have been described,^{3–6} including in Indiana with estimates indicating that 43% of residents infected with SARS-CoV-2 show no symptoms.⁷ Further, viral transmission from asymptomatic individuals has been confirmed.^{3–6} In fact, one mathematical model suggests approximately 50% of new SARS-CoV-2

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infections originate from individuals without symptoms at the time of transmission, this includes individuals who are presymptomatic and those who will remain asymptomatic over the course of their infection.⁸ Despite the now well-documented asymptomatic proportion of infection and infectivity of asymptomatic infections, there is a dearth of longitudinal studies documenting the clinical experience of initially asymptomatic infections. A narrative review of studies of asymptomatic SARS-CoV-2 infections noted that in four of five studies with longitudinal data (of variable length), only a fraction of persons originally asymptomatic subsequently developed symptoms.⁹ A better understanding of asymptomatic infections is a critical knowledge gap and an increasing point of interest in curbing the pandemic.

The Asymptomatic novel CORonavirus iNFection (ACORN) study was designed to study the prevalence of SARS-CoV-2 infection in asymptomatic adults of the Indianapolis metropolitan area over a 6-week period between April 7 and May 9, 2020. Over this period, 91 of 2953 individuals (3.1%) initially presenting as asymptomatic for coronavirus disease 2019 (COVID-19) based on the absence of new or worsening cough, new or worsening shortness of breath, or fever in 7 days prior tested positive for SARS-CoV-2.¹⁰ In an effort to better understand the course of infection in these initially asymptomatic infections, participants were followed to inquire for the development of symptoms, and investigate the duration of positive test results by repeat nasopharyngeal swabs. Interim results have been previously reported for 81 of the SARS-CoV-2 positive participants.¹⁰ Here we report the final results from the ACORN observational study, with an emphasis on the longitudinal follow-up of initially asymptomatic participants testing positive for SARS-CoV-2.

2 | METHODS

2.1 | Study design and participants

ACORN is a cross-sectional, community-based, observational study of adults presenting asymptomatic for COVID-like illness, with a nested longitudinal study for participants that test positive. Asymptomatic upon study enrollment was defined as the self-reported absence of fever ($\geq 100^{\circ}\text{F}$), new onset or worsening cough, and new onset or worsening of shortness of breath within the previous week.

Study design, patient eligibility, and statistical analysis have been previously reported.¹⁰ Briefly, nasopharyngeal swabs were collected in-person by trained personnel at a drive-through testing facility. Test results were available within 1–3 days of sample collection. Those who tested positive were contacted by trained medical professionals approximately 14 days after the sample resulting in a positive test was collected and given a structured questionnaire to query symptom development since the time of the test. Symptoms specifically queried were as follows: fever, cough, shortness of breath, chills, fatigue or muscle aches, sore throat, headache, GI symptoms, loss of smell or taste. If symptoms were reported, date of first symptom onset was asked. Severity of symptoms was not queried. Participants were asked if they had been hospitalized for COVID-related reasons. Open report of other

symptoms was allowed. Additionally, during the follow-up contact querying symptom development, the participant was invited to return for a repeat reverse transcription-polymerase chain reaction (RT-PCR) test. A maximum of 3 repeat nasopharyngeal swabs at approximately 2-week intervals from initial test were offered until the result was negative. As such, the study activities (i.e., RT-PCR testing and follow-up telephone call, if positive) were repeated at 14-day intervals for anyone consenting to participate in repeat testing until the participant either tested negative, completed three repeat tests, or was lost to follow-up.

The study protocol (including the protocol addendum for repeat testing) was approved by an external IRB (WIRB-Copernicus) and performed in compliance with relevant regulations and in accordance with the ethical standards of the Declaration of Helsinki. All participants provided informed consent.

2.2 | Specimen collection and RT-PCR for SARS-CoV-2

Specimens were collected at a drive-through testing facility by trained personnel according to Centers for Disease Control and Prevention (CDC) recommended methods for nasopharyngeal swabs.¹¹ The SARS-CoV-2 qualitative RT-PCR test was analytically validated using CDC primer and probe set(s) in the Clinical Diagnostics Laboratory, Eli Lilly and Company, that is a Clinical Laboratory Improvement Amendments of 1988, 42 U.S.C. §263a certified high-complexity laboratory. The validation was designed to meet the Food and Drug Administration (FDA) Emergency Use Authorization for SARS-CoV-2 PCR testing¹² and submitted to both the FDA and Indiana State Department of Health for review. The assay validation demonstrated sensitivity and specificity of 100% and a limit of detection of 1000 copies/ml. The cycle threshold (C_t) during RT-PCR testing refers to when the detection of viral amplicons occurs and is inversely proportional to the amount of RNA present. A lower C_t value indicates a greater quantity of viral RNA in the sample. The threshold for positivity was a C_t value for either viral N1 or viral N2, or both, that was less than 40. Furthermore, a board-certified pathologist reviewed all amplification curves before results were released. This review was particularly important for the cases with low viral load (high C_t) where evaluation of the curve in the context of the control gene may lead to pathologist override or request for repeat running of RT-PCR.

3 | RESULTS

3.1 | Prevalence results

As previously reported,¹⁰ 91 of 2953 participants asymptomatic for SARS-CoV-2 tested positive initially over the 6-week period of study enrollment (3.1%; 95% confidence interval, 2.5%–3.7%). The weekly point prevalence was generally consistent over the course of the 6 weeks (Figure 1).

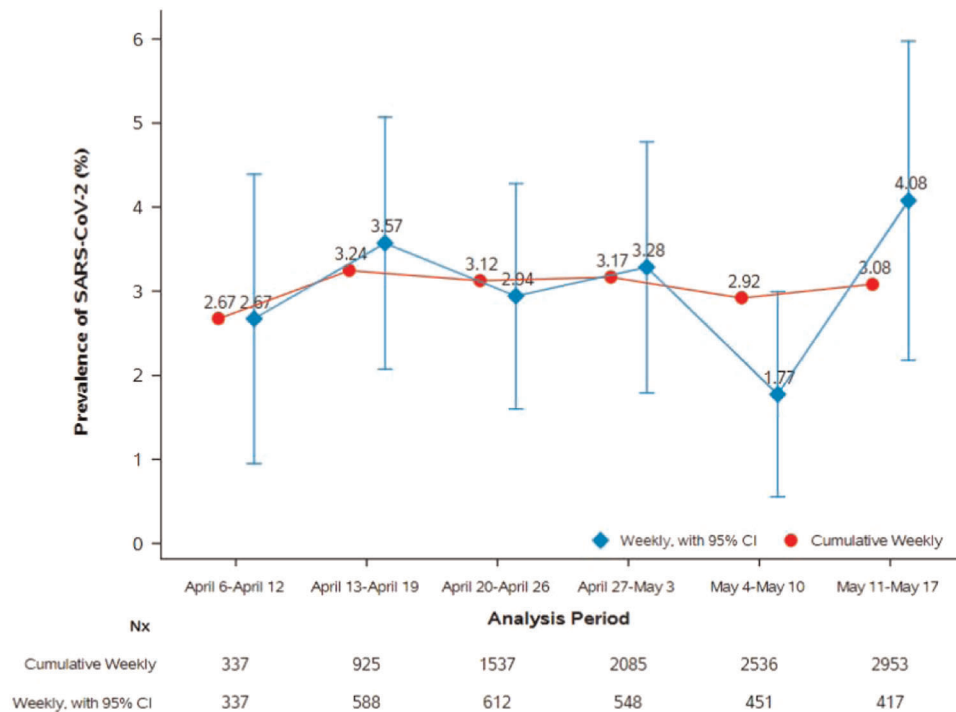


FIGURE 1 Weekly and updating cumulative prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within the Asymptomatic novel CORonavirus iNfection (ACORN) study of asymptomatic individuals during study enrollment

An analysis was conducted to assess the sensitivity of the prevalence estimate to the definition of “asymptomatic,” which was absent new or worsening fever, cough, or shortness of breath in the 7 days before enrollment. There were a total of 492 participants that reported at least one other baseline symptom (either chills, fatigue or muscle aches, sore throat, headache, GI symptoms, or loss of smell or taste), among which 13 ($N = 91$; 14.3%) tested positive and 479 ($N = 2862$; 16.7%) tested negative.¹⁰ In an analysis removing all 492 participants reporting any other symptom at study enrollment, the prevalence of SARS-CoV-2 infection remained consistent at 3.2% (78 of 2461 participants).

Demographics and health characteristics for the participants of the ACORN study were previously published.¹⁰ In summary, the average age of the overall study cohort was 49.6 years old (range 18–88), 58.9% female, and 89.8% White. When qualitatively comparing those participants testing positive to the overall sample, participants positive for SARS-CoV-2 were slightly younger (average age was 48.1 years), more often female (63.7%), and slightly lower proportion White (86.8%). Over 95% of both the entire study cohort and those testing positive self-reported their overall general health as either excellent or good. Hypertension was the most prevalent pre-existing condition (approximately 20% in overall cohort and among those testing positive), approximately a quarter of participants reported former smoking (24.2% in overall cohort and 25.3% among those testing positive), and nearly a third of the cohort reported a height and weight resulting in body mass index ≥ 30 (kg/m^2) (27.4% in overall cohort and 29.7% among those testing positive).

3.2 | Follow-up among participants positive for SARS-CoV-2

Among the 91 participants testing positive for SARS-CoV-2, 86 completed a follow-up interview at 14 days to query for development of symptoms (Figure 2). Among those completing the Day 14 follow-up survey, 18 (20.9%) reported developing any new symptom after their initial positive test. There was 1 additional participant who reported a new symptom at the Day 28 follow-up, new onset allergy-induced asthma was reported. In total, among all participants who completed at least the Day 14 follow-up, 19 (22.1%) developed at least one symptom at any time after the initial positive SARS-CoV-2 test result. The median number of days to symptom development after their initial positive test result was 6 (range 1–29 days). No participants reported a SARS-CoV-2 related hospitalization during the follow-up period. The most frequently reported symptoms were fatigue or muscle aches (10.5%), headache (9.3%), fever (5.8%), shortness of breath (5.8%), and open report of other symptoms (5.8%). Of the remaining symptoms within the structured questionnaire, each was reported by ≤ 4 participants (Table 1). While severity of symptoms was not systematically collected, the experience noted by the medical professionals conducting follow-up interviews was that for the participants reporting symptom onset, symptoms were generally mild in nature.

Of the 86 participants contacted at Day 14, 78 submitted a repeat nasopharyngeal swab for RT-PCR testing, and 17 (21.8%) remained positive. The 17 participants that tested positive at Day 14 were contacted again at approximately 28 days from initial positive

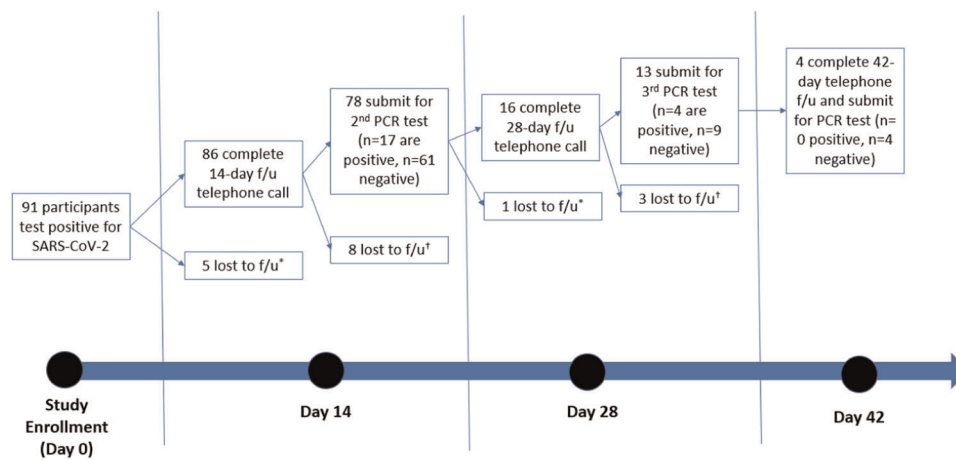


FIGURE 2 Disposition of participants testing positive at enrollment into the ACORN study. ACORN, asymptomatic novel coronavirus infection

test. Thirteen of these 17 participants submitted for a third nasopharyngeal swabs for RT-PCR testing, and 4 (30.7%) remained positive. All four participants that tested positive at Day 28 were contacted again at approximately 42 days from the initial positive test. All four participants who submitted for a fourth and final nasopharyngeal swab had negative results at approximately 42 days

from the initial positive test. Last patient visit occurred June 15, 2020.

3.3 | Ct values from RT-PCR assay for SARS-CoV-2

The RT-PCR C_t values from positive nasopharyngeal swab samples at study entry ($n = 91$), and the C_t values from those initially positive participants that continued to test positive at Day 14 or 28, are displayed in Figure 3. The median (interquartile range) C_t at the time of study enrollment was 36.2 (34.8–37.4). C_t distribution from the initial RT-PCR test was consistent when stratifying participants by those who remained asymptomatic at the Day 14 follow-up contact and those who were presymptomatic (Figure S1). The median C_t was consistent among participants testing positive at Day 14 ($n = 17$) and Day 28 ($n = 4$), 35.9 and 35.4, respectively, with individual variability across timepoints (Figure S2).

4 | DISCUSSION

Results from the ACORN study provide estimates of SARS-CoV-2 infection prevalence in the Indianapolis area among asymptomatic people between April 7 and May 9, 2020. Importantly, this final analysis provides in-depth profiling on symptom development among an initially asymptomatic cohort, duration of positive RT-PCR tests for SARS-CoV-2 infection among those who have tested positive, and information regarding viral load, as determined by C_t values.

The ACORN study is unique in that it presents the clinical course and serial testing (i.e., nasopharyngeal swab and RT-PCR C_t) data from a community-based sample of initially asymptomatic people with SARS-CoV-2 infections. This contrasts with many currently published studies on SARS-CoV-2 infections that focus on patients (either symptomatic or asymptomatic) from either isolation hospitals, nursing homes, or other selected patient pools based on where testing resources are directed.

TABLE 1 Summary of symptoms reported to develop at any time after initial SARS-CoV-2 positive test, among participants with at least 1 follow-up contact at Day 14 ($N = 86$)

Symptom/healthcare resource	All subjects ($N = 86$) n (%)
Subjects with ≥ 1 symptom	19 (22.1%)
Fever ^a	5 (5.8%)
Chills	3 (3.5%)
Fatigue or muscle aches	9 (10.5%)
Sore throat	4 (4.7%)
Cough	3 (3.5%)
Shortness of breath	5 (5.8%)
Headache	8 (9.3%)
GI symptoms ^b	4 (4.7%)
Loss of smell or taste	3 (3.5%)
Other symptoms ^c	5 (5.8%)
Hospitalization related to SARS-CoV-2 infection	0 (0%)

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aTemperature ≥ 100 degrees Fahrenheit.

^bNausea, vomiting, or diarrhea.

^cFive subjects reported other symptoms. The “other” symptoms reported by each of subject are as follows: chest pain, tightness in chest; nasal and head congestion; loss of appetite; chest tightness; allergy-induced asthma.

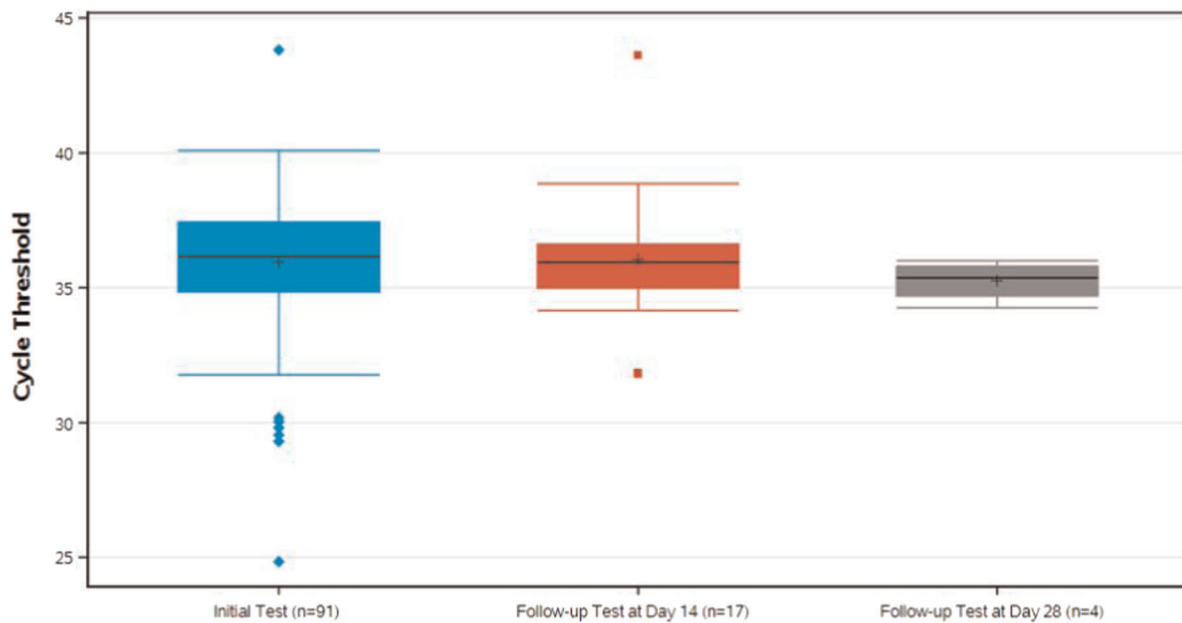


FIGURE 3 Distribution of SARS-CoV-2 RT-PCR cycle threshold among participants testing positive at study enrollment, and subsequent follow-up tests. RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Over three-quarters of those testing positive for SARS-CoV-2 remained asymptomatic, and among those that did develop symptoms, they were generally mild and nonspecific. It is worth noting that the classification of presymptomatic in this study was rather generous, in that a person who reported development of any symptom was considered. As such, it is possibly an overestimate to state 22% were presymptomatic. Many, if not most, of the participants would not have known about their viral status if not participating in this study. The low percentage of participants reporting symptoms could be reflective of a selection bias resulting from the study design (a convenience sample of persons with knowledge of and access to a personal vehicle for drive-through testing) and cohort characteristics (relatively young, mostly White, and with over 95% self-reporting good or excellent health). Despite these possible limitations, a study from Vò, Italy, where nasopharyngeal swabs were collected on nearly every resident, reported that none of the 29 asymptomatic persons testing positive developed any symptom of COVID-19 over a 14-day period.⁴ In another report which included 39 asymptomatic infections among a cohort of Greek citizens repatriating from various countries, 35 (87.5%) remained asymptomatic about 14 days later.⁹ While longitudinal data on persons with asymptomatic infections are scarce, these two reports, combined with longitudinal results presented here from the ACORN study, suggest that a large majority of asymptomatic infections remain free of symptoms for the duration of their viral shedding.

COVID-19 symptoms are poor markers for SARS-CoV-2 infection, with one study reporting a positive predictive value of only 10% for seemingly specific symptoms, such as fever, cough, or loss of taste or smell, and even less predictive when considering any symptom.¹³ Given symptoms alone are a poor marker for infection, and the low

percentage of initially asymptomatic participants that develop any kind of symptom, it is clear that reliance on development of symptoms over the course of infection is not a complete strategy for identifying persons with SARS-CoV-2 infections.

There is a dearth of longitudinal, community-based studies of asymptomatic SARS-CoV-2 infection from which to understand the duration of viral shedding for asymptomatic infections (as determined by RT-PCR), and the period which transmission of infection may occur. Among the ACORN study participants returning for repeat tests approximately 14 days after initial test ($n = 78$), a large majority (78.2%) were no longer shedding detectable virus in their nasopharyngeal samples. There were four participants that continued to test positive through RT-PCR approximately 28 days after their initial test, but all four returned negative test results approximately 42 days after initial test. Given the community-based, cross-sectional nature by which participants were originally identified, the index date of infection is not known. Thus, this analysis of duration of viral shedding in the ACORN study has limitations. Persistent positive RT-PCR results have been described in other studies as well, including the testing of crew members of the U.S.S. Theodore Roosevelt.^{14,15}

The viral load was generally low with the ACORN study cohort of asymptomatic infections, as indicated by higher C_t values. No notable differences in C_t were demonstrated for those that were asymptomatic versus presymptomatic. This could be because those participants classified as presymptomatic were reporting mild and nonspecific symptoms. Further, among participants with persistent viral shedding as noted by persistent positive results from RT-PCR, the viral load remained relatively consistent, for as long as they tested positive. However, viral shedding as measured by C_t from the PCR does not equate to infectiousness. While patients were

counseled on self-isolation, full contact tracing was not conducted; therefore, transmission was not assessed.

There is a need to better understand transmission of the virus from those with SARS-CoV-2 who remain asymptomatic or become mildly symptomatic. A recognized limitation of RT-PCR is that the test will identify virus over the course of an infection, including after a person is infectious. However, the window for transmission of the virus to another person is not known. A recent review noted that asymptomatic persons can transmit SARS-CoV-2 for an extended period, perhaps longer than 14 days.⁹ While there are reports correlating transmission potential with higher viral loads (i.e., low C_t values), SARS-CoV-2 has also been cultured from persons with low viral loads.^{16,17}

There are two noted limitations to the classification of “asymptomatic” in the ACORN study. First, the presence or absence of symptoms (both for determining eligibility and at follow-up) was based on self-report and not confirmed in-person or via medical records. It is possible that to obtain a test, people were mis-reporting their symptoms, which would then overestimate the prevalence among an asymptomatic population. Second, asymptomatic status for determining study eligibility was defined by the absence of the three most prominently recognized symptoms of COVID-19 at the time the study was developed, notably fever, cough, shortness of breath. Over the course of the study, the CDC updated the symptom profile for COVID-19 to include fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea.¹⁸ While these additional symptoms were not considered exclusion criteria for enrollment, most were queried during the enrollment questionnaire.¹⁰ The prevalence estimate among an asymptomatic cohort was not sensitive to these definitions of “asymptomatic” and the minimal reporting of symptom development in baseline suggests that neither of these limitations influenced the presented estimate of prevalence within an asymptomatic cohort.

5 | CONCLUSION

Asymptomatic SARS-CoV-2 infection was determined to have a point prevalence of 3.1% in a community-based study. Most participants remained asymptomatic, although 22% developed symptoms (generally mild) post-enrollment. These findings reinforce the probable role of silent SARS-CoV-2 infections in community transmission, and that reliance on symptom development will miss a large proportion of infections. Broad testing programs, not limited to individuals presenting with symptoms, are critical for identifying persons with SARS-CoV-2 infection and ultimately slowing transmission.

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CONFLICT OF INTERESTS

All authors are employees and shareholders of Eli Lilly and Company.

AUTHOR CONTRIBUTIONS

All authors participated in the drafting and critical revision of the manuscript. All authors approved the final submitted version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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