

Quantifying the prevalence of SARS-CoV-2 long-term shedding among non-hospitalized COVID-19 patients

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With anecdotal reports of viral shedding from COVID-19 patients for several weeks, there is a need to quantify the prevalence of long-term SARS-CoV-2 shedding. Here, we characterize the temporal distribution of diagnostic SARS-CoV-2 PCR outcomes from nasopharyngeal swabs and associated EHR-derived features over two months for 874 COVID-19 patients with longitudinal data. Among a cohort of 379 COVID-19 patients with at least one positive follow-up SARS-CoV-2 PCR test, 53 patients remain SARS-CoV-2-positive after four weeks of initial diagnosis. Surprisingly, a majority of COVID-19 patients with long-term viral shedding are not hospitalized (40 of 53 patients), and have no enrichments among symptoms, demographics, or medical history. In a cohort of 370 COVID-19 patients that transition to a confirmed negative status, the upper bound of viral shedding duration has a mean of 21.2 days with standard deviation of 9.3 days. Of the 81 PCR-confirmed COVID_{pos} patients who have undergone serologic testing, 68 patients have developed anti-SARS-CoV-2 IgG to date, with a mean upper bound of time to seroconversion of 38.1 days (95% C.I. = 35.2-41.1 days). Given that SARS-CoV-2 PCR testing may detect replication incompetent virus and that serologic tests do not imply neutralizing immunity, we suggest that the development of novel assays for measuring infectious viral load in non-hospitalized long-term shedders may help mitigate community transmission. This study motivates a platform that can link longitudinal diagnostic and serologic testing with real-time epidemiological data, towards proactively identifying and managing emerging hotspots of COVID-19.

Introduction

As COVID-19 continues to rage globally with over 5 million confirmed infected individuals and 300,000 deaths¹, the world is grappling with the dual challenge of stemming the tide of the current pandemic and planning for reopening the economies in the post-COVID phase. Currently, there are over a million patients that have recovered from COVID-19¹, and some governments have suggested that antibody-based tests in recovered individuals can be used as the basis for an “immunity passport”² to travel or return-to-work assuming that they are protected against re-infection. However, there are also emerging reports of viral shedding for many days post-recovery, as evidenced from PCR tests on stool samples³ and recurrent SARS-CoV-2-positive

PCR tests in “cured” patients⁴. The lack of quantification of infection duration, viral shedding, and potential for transmission necessitates longitudinal monitoring of viral clearance in COVID-19 patients. Such an analysis has the potential to help inform epidemiological strategies to help ‘flatten the curve’ within communities most affected by the ongoing pandemic, and also help shape the evolving guidelines from states and governments regarding the duration of self-quarantining among COVID-19 patients⁵.

Results

Between February and May 2020, 74,993 individuals underwent a SARS-CoV-2 PCR test at the Mayo Clinic hospitals in Minnesota, Arizona and Florida, or the associated Mayo Clinic Health System (**Figure 1a**). 12,445 individuals (16.6%) were subjected to the test more than once, with most of these individuals ($n = 11,474$) subjected to 2 or 3 PCR tests each (**Figure 1a**). Of all the individuals tested, 2,239 patients tested SARS-CoV-2-positive at least once during the study period (henceforth, COVID_{pos}) (**Figure 1b**). The age distributions in the context of hospitalization, intensive care unit (ICU) admission, and mortality status are shown for COVID_{pos} patients in **Figure 1c-f**. Notably, 45% of the COVID_{pos} patients in this study are in the age group of 19-40. The pattern of increased hospitalization, ICU admissions and death in the elderly compared to the younger populations is consistent with previous studies of COVID-19 patient demographics⁵⁻⁷.

Among the 2,239 COVID_{pos} patients, 874 patients (39%) took two or more PCR tests, and 379 patients (16.9%) had at least two SARS-CoV-2-positive tests (**Figure 1b,g**). It is noted that 537 of 2,239 COVID_{pos} patients were administered at least two additional PCR tests after their initial diagnostic test, with 108 patients oscillating from SARS-CoV-2-positive to SARS-CoV-2-negative and back to SARS-CoV-2-positive status one or more times (**Figure 1h**).

The availability of these longitudinal PCR test results and their associated Electronic Health Record (EHR), provides an excellent opportunity to systematically quantify the duration of COVID-19 infection. Specifically, we aimed to quantify for each patient (1) a *lower bound of infection duration*, defined as the time in days between the first and last positive SARS-CoV-2 PCR tests, and (2) an *upper bound of infection duration*, defined as the time in days between the first positive PCR test and the second negative PCR test after which there are no further positive PCR tests (**Figure 2a**). The lower bound captures the most intuitive infection duration. Nevertheless, our quantification of the upper bound is motivated by recent reports of high false-negative rates for SARS-CoV-2 PCR tests⁸, our own observation of oscillations in serial PCR outcomes (**Figure 1h**), and the requirement for healthcare workers to receive negative PCR results on two consecutive days to meet the CDC “Return to Work” criteria⁵.

COVID-19 patients whose lower bound of infection duration is greater than four weeks (28 days) are hereafter referred to as patients displaying ‘long-term shedding’. For the 370 COVID_{pos} patients that switched to a confirmed negative status, i.e. two negative SARS-CoV-2 PCR tests following last positive SARS-COV-2 test, the distribution of the upper bound of infection duration has a mean of 21.2 days and a standard deviation of 9.3 days (**Figure 2b**).

Of the 379 COVID_{pos} patients with at least two positive tests, interestingly, 53 patients (14%) display long-term shedding (**Figure 2c**), and strikingly the majority of these patients are not hospitalized. Based on the analysis of all the available positive SARS-CoV-2 IgG antibody tests (n = 68 patients), the upper bound of time to IgG-seroconversion from initial PCR diagnostic testing has a mean of 38.1 days (95% confidence interval: 35.2-41.1 days; **Figure 3a**). Here, we consider an upper bound rather than a definitive time to seroconversion as each patient likely experienced IgG seroconversion prior to the testing date. Based on the limited longitudinal ‘real world evidence’ available for IgG seroconversion, this upper bound is the best estimate we are able to obtain at this juncture. Despite this caveat, a few patients are noted to turn sero-positive around 10 days post their initial SARS-CoV-2-positive PCR diagnosis date. The finding that the time to IgG seroconversion is shorter than the lower bound of infection based on PCR tests, suggests that COVID-19 patients can continue to shed virus while generating antibodies (**Figure 3b**). While it is unclear whether a SARS-CoV-2-positive PCR test is indicative of replication-competent virus^{9,10} and likewise whether SARS-CoV-2 IgG antibody testing is reflective of immunity, the assessment of viral loads and antibody titers would provide additional insights. For instance, such additional lab tests would help inform whether current CDC guidelines of 10 days self-quarantining for asymptomatic patients is broadly satisfactory, including for patients with long-term SARS-CoV-2 shedding⁵.

Examining the factors that may have warranted follow-up PCR testing that facilitated our identification of long-term shedding does not provide any obvious explanation. For instance, patients with long-term shedding do not seem to be enriched for health care workers or have any discernible underlying condition. Despite social distancing norms and rigorous adherence to self-quarantining, some of the asymptomatic COVID-19 patients with protracted SARS-CoV-2-positive PCR tests may indeed be increasing the odds of unintended community transmission.

In order to better understand whether COVID-19 patients with long-term shedding display any distinctive symptoms, we defined a control cohort of COVID-19 patients with an *upper bound of infection duration* between 1 to 13 days. We compared the COVID-19 patients displaying long-term shedding with this control cohort by analyzing 269 commonly-occurring clinical features out of over 15,000 that were extracted from various structured databases constituting the COVID_{pos} patient’s Electronic Health Records (EHRs) between the February-May 2020 study period. These EHR databases examined include, but are not limited to, diagnosis, ICD codes, medication history, immunization records, procedures, vitals, lab tests, and demographics (*see Methods*). Surprisingly, we do not find any significant distinguishing clinical features for COVID-19 patients who are long-term shedders. While this preliminary observation has to be monitored as more COVID-19 patients’ longitudinal data are incorporated, at this juncture, it appears that the majority of long-term shedders may not have significant symptomatology that could have prompted their clinical follow-up.

Discussion

A recent study from China of 74 COVID_{pos} patient’s fecal samples and respiratory swabs observed SARS-CoV-2-positive swabs with a mean duration of 15.4 days and standard deviation of 6.7 days from the first symptom onset¹¹. In this study, we have shown that COVID-19 patients

with long-term shedding are predominantly not hospitalized and potentially asymptomatic, thus underlining the importance of understanding the temporal dynamics of viral load, the duration of infectivity, and the likelihood of community transmission. Studies focusing on the temporal profiles of viral shedding suggest that the viral loads are highest at the symptom onset which decreases monotonically towards detection limit around day 21^{10,12} and that live virus could no longer be cultured after day 8, leading to the hypothesis that SARS-CoV-2 infectiousness may decline from the time of symptom onset^{9,10}. Whether such experimental results are generalizable to all COVID-19 patients with long-term shedding is an important follow-up question stemming from this study. Although the SARS-CoV-2-positive PCR tests used as the yardstick for estimating the prevalence of long-term shedding in this study may by no means causally implicate replication-competent virus, the presence of viral RNA for several weeks from initial infection certainly warrants longitudinal monitoring of the viral load. Nonetheless, the question still remains as to why some COVID_{pos} patients shed viruses and viral RNA for far longer than other COVID_{pos} patients. Additional research into the immunological, microbiome, environmental, immunization, and other factors associated with long-term shedding is necessary to help rationalize our clinical observations.

It may be noted that the clinical sensitivity of SARS-CoV-2 PCR tests has been debated to some extent¹³, and certainly there are anecdotal examples from our own clinic experience where critical ill COVID-19 patients can switch from a COVID_{pos} status to a COVID_{neg} status within a short period of time. To robustly enable scientific assessment of the sensitivity of our PCR tests, we summarized the entire pattern of serial PCR outcomes across the 2,239 COVID-19 patients in this study. This analysis shows that a vast majority of the COVID-19 patients subjected to PCR produce consistent outcomes, as determined by multiple contiguous PCR tests resulting in the same outcome. There is a small minority of COVID-19 patients where aberrant switching of PCR outcomes is indeed observed, and the reasons for this clinical observation that compromises sensitivity of PCR testing as a diagnostic gold standard remain speculative at this juncture.

Several factors could influence viral shedding. Replicative fitness of a given virus is one of them. For instance, in HIV, not all viruses replicate equivalently, and differences are due to polymorphisms in different genes¹⁴. For SARS-CoV-2 there are reports of different polymorphisms impacting disease or transmissibility (e.g. D164G)¹⁵. Another factor is timing and quantum of immune response. For example, given the role of IFN response in viral shedding¹⁶, early IFN response is likely to be beneficial and reduce shedding, whereas late IFN response may be deleterious and delay clearance. Another potential factor is T cell response¹⁷. When T cells express high levels of different effector mechanisms (e.g. Perforin/granzyme B, IFN, FasL/ TRAIL etc), they work better than if they produce only one effector pathway.

Our findings raise important additional follow-up questions. Recent studies have identified coagulation associated changes in COVID-19 patients^{18,19}. In the wake of these findings, it becomes important to examine the rate of change of coagulation signals (e.g. by longitudinal lab testing of platelet count, fibrinogen levels, d-dimer values) and immune cells (e.g. neutrophils, monocytes, basophils, lymphocytes) in COVID-19 patients with long-term shedding versus patients who more rapidly eliminate the virus. It is also intriguing to examine how the duration of shedding correlates to the rate of seroconversion and the presence of effective humoral immunity

as measured by neutralizing antibodies. While our study certainly calls for more prolonged longitudinal testing of viral RNA as well as live viruses, it remains to be seen how practical this is, given reports of insufficient availability of test kits²⁰.

Given the significant value of longitudinal SARS-CoV-2 PCR testing, efforts are underway to develop an epidemiological tracking platform that integrates the SARS-CoV-2 testing data at the county-level across states that have published this information (**Figure 4**). One of the applications in this platform ('Measures app') enables measuring geographical and temporal trends of SARS-CoV-2 test positivity - i.e. positive SARS-CoV-2 PCR tests as a fraction of the total SARS-CoV-2 PCR tests conducted by each county. Given our identification of long-term shedding across multiple counties in the state of Minnesota, such a *Precision COVID-19 platform* can aid in the detection of "hot spots" of SARS-CoV-2 infectivity and guide the appropriate allocation of PPE resources as well as diagnostic kits to get ahead of community transmission proactively.

Our finding of significant SARS-CoV-2 long-term shedding in COVID-19 patients suggests that prolonged monitoring of viral loads in COVID-19 patients together with sustained periods of low SARS-CoV-2 test positivity, could jointly help inform when to reopen each county's operations. Ultimately, understanding the personalized factors underlying SARS-CoV-2 long-term shedding by some COVID-19 patients will be important to better inform such return-to-work strategies that are underway within large enterprises, as well as across states and countries.

Methods

SARS-CoV-2 diagnostic tests conducted by Mayo Clinic hospitals and health system

In regards to PCR methodology, for Mayo Clinic patients seen in the Rochester MN hospital, a pair of SARS-CoV-2 diagnostic tests were employed^{21,22}. The Roche Cobas diagnostic test was employed by the Mayo Clinic's Florida hospitals, and the Abbott diagnostic test was used by the Mayo Clinic's Arizona hospitals²³. These SARS-CoV-2 PCR tests used in this study do amplify different segments of the viral genome, but are considered largely equivalent from the perspective of their analytical performance. The LOINC code of the SARS-CoV-2 IgG test administered is 94563-4²⁴.

Statistical analysis of longitudinal SARS-CoV-2 PCR results

The features considered in the analysis to discriminate the COVID-19 long-term shedding patients include all structured entities from the EHR, including but not limited to demographics, diagnosis, ICD codes, medication history, immunization record, procedures, vitals and lab tests. Any feature which is enriched significantly towards either shorter durations (less than 14 days between first positive to second negative test, as depicted in **Figure 2b**) or longer durations (greater than or equal to 28 days between first positive to recent/final positive test, i.e. 'long-term shedders' as depicted in **Figure 2c**) was noted down. During the observation period (n = 116: 53 long-term shedding patients; 63 control patients), there were 269 EHR-derived features that were

considered, including potentially prior to each patient's COVID-19 diagnosis. The 2-proportion z-test p-value (after BH adjustment for multiple hypothesis correction) was used to assess the differences of each feature between the long-term shedding patients and the control cohort, defined as those COVID-19 patients with an *upper bound of infection duration* between 1 to 13 days. The procedure was as follows:

1. Filter by features which are present in overall at least 10% of the patients we're looking at.
2. It's possible that there is a bias of more overall features towards the long-term or control cohort. We are not interested in this bias. To account for this, for each feature, we compute the "baseline" proportion difference, i.e. the weighted mean proportion of long-term shedders that are positive for that feature minus the weighted mean proportion of control cohort which is positive for that feature. Call this baseline difference O (we have one such O for each feature).
3. Perform a 2-proportion z-test for whether the difference between feature-positive rate in the long-term cohort and feature-positive rate in the control cohort is significantly different from the baseline O .
4. Adjust these p -values for multiple hypotheses using the Benjamini-Hochberg procedure (with FDR controlled at 0.1 level).

We repeated the above procedure for slightly different underlying data as well; in particular, we re-ran on the following variants:

- i. We filtered to look only at patients who were not hospitalized (as those would be of most concern).
- ii. Each binary feature (phenotype, lab test, etc) occurred at a particular day in the patient's record. We filtered by only those features which occur 0, 21 or 28 days following diagnosis.
- iii. Variations (i) and (ii) together

Statistical analysis employed in the Precision COVID-19 platform

The following metrics are used as part of this epidemiological tracking application:

Total Population: number of people that live in a given territory. This is based on USA Census data.

Tests: number of patients tested for SARS-CoV-2 (PCR testing) to date. This information is based on Mayo Clinic Labs testing.

Positive Tests: number of patients tested for SARS-CoV-2 (PCR testing) that have been positive to date.

%Positive: fraction of patients tested (PCR testing) that have been positive for SARS-CoV-2 to date. The color schema used was based on worldwide benchmarks using South Korea as a positive reference and New York as a negative reference.

Relative Testing: the number of tests per capita in a territory divided by the number of tests per capita in the region where that territory belongs to. For example, a county with a Relative Testing of 2.0 means that such county has performed twice as many tests per capita relative to the entire state.

Cases (Gov't): this is the number of SARS-CoV-2 positive patients in the territory as reported by each government or state agency.

Relative Incidence: number of SARS-CoV-2 cases per capita in a territory (as reported by government/state agencies) divided by the number of cases per capita in the region. For example, a county with a Relative Incidence of 2.0 means that such a county has had twice as many SARS-CoV-2 cases per capita relative to the entire state.

Testing-to-Incidence Ratio: this is the ratio between Relative Testing and Relative Incidence at the county level.

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Conflict of Interest Statement

One or more of the investigators associated with this project and Mayo Clinic have a Financial Conflict of Interest in technology used in the research and that the investigator(s) and Mayo Clinic may stand to gain financially from the successful outcome of the research. The title of the project is – IRB 20-003334: “CDAP – Study of COVID-19 patient characteristics with augmented curation of Electronic Health Records (EHR) to inform strategic and operational decisions”. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and is being conducted in compliance with Mayo Clinic Conflict of Interest policies.

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Figure Legends

Figure 1. Distributions of (a) number of PCR tests per individual, (b) number of PCR tests taken by COVID_{pos} patients, (c) age of COVID_{pos} patients, (d) age of hospitalized COVID_{pos} patients, (e) age of ICU-admitted COVID_{pos} patients, (f) age of deceased COVID_{pos} patients, (g) the number of patients by sequence of SARS-CoV-2 PCR positive and negative results, and (h) the number of switches between COVID_{pos} and COVID_{neg} status in longitudinal testing of COVID_{pos} patients; box indicates the count of patients that switched from COVID_{pos} to COVID_{neg} and back to COVID_{pos} status at least once. (c-f) Compared to the overall COVID_{pos} patient population that includes a considerable proportion of 0-40 year old individuals (n = 2239, mean = 41.4 years, standard deviation = 19 years, median = 39 years), there is a shift to the older aged population among the

hospitalized patients ($n = 190$, mean = 58.2 years, standard deviation = 18.9 years, median = 60 years), ICU patients ($n = 77$ patients, mean = 58.9 years, standard deviation = 14.8 years, median = 59 years), and deceased patients ($n = 30$ patients, mean = 83.5 years, standard deviation = 9.3 years, and median = 86.5 years).

Figure 2. Distribution of the COVIDpos patients by **(a)** duration between the day of diagnosis to first negative test after last positive test. **(b)** duration between the day of diagnosis to the last positive test.

Figure 3. Distribution of upper-bound of the duration to convert to sero-positive status based on SARS-CoV-2 IgG test and comparison to COVID_{pos} status based on SARS-CoV-2 IgG antibody test. (a) Histogram of duration (in days) between the day of diagnosis based on SARS-CoV-2 PCR test and day of seropositive status based on SARS-CoV-2 IgG test. (b) Comparison of sero-positive status (based on antibody test) and COVIDpos status (based on PCR test). Cases that are sero-positive and shedding virus are boxed.

Figure 4. The 'Precision COVID-19' platform that is being developed for real-time epidemiological surveillance of positive SARS-CoV-2 PCR tests conducted as a fraction of the total SARS-CoV-2 PCR tests conducted that day within each county is visualized herein.

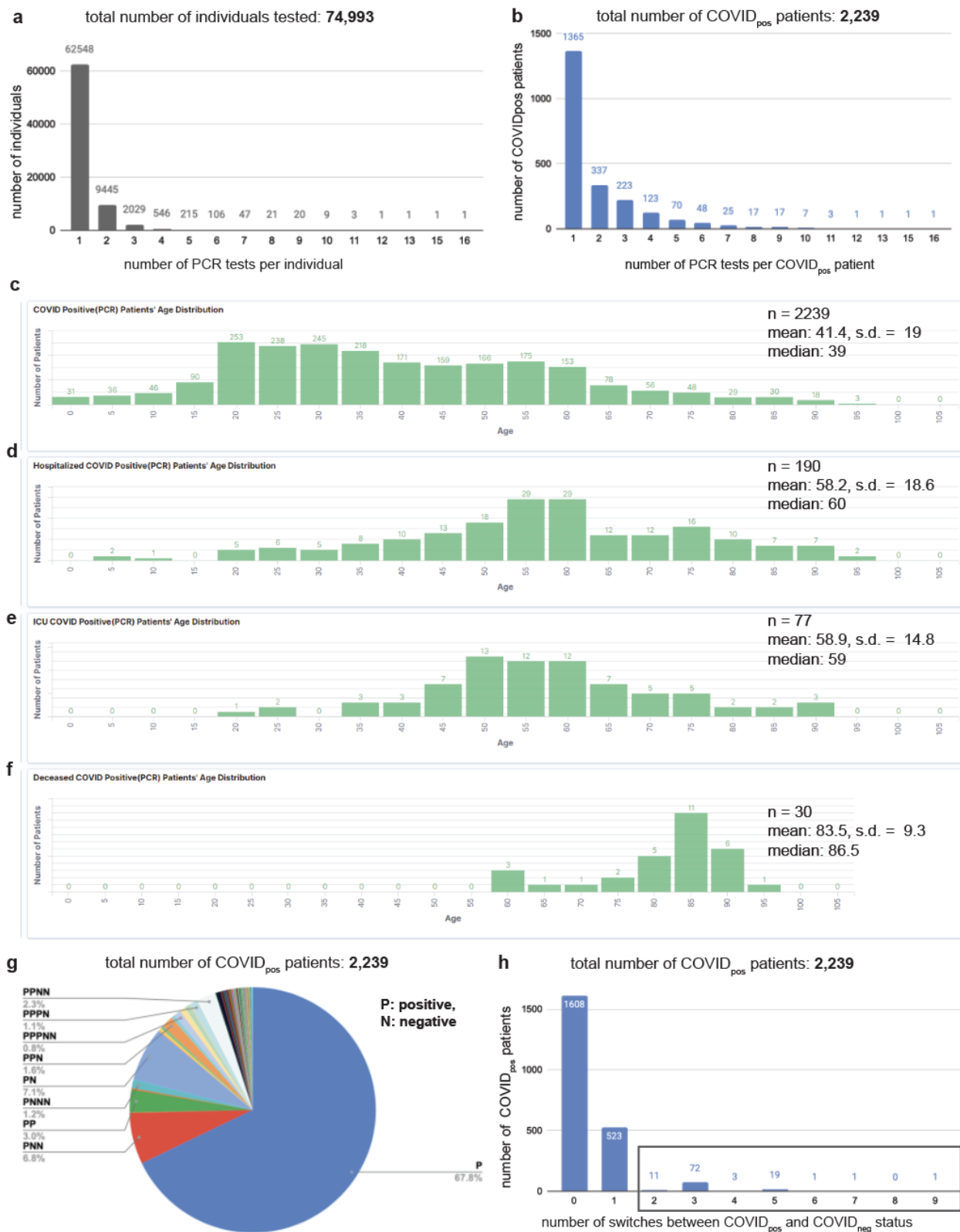
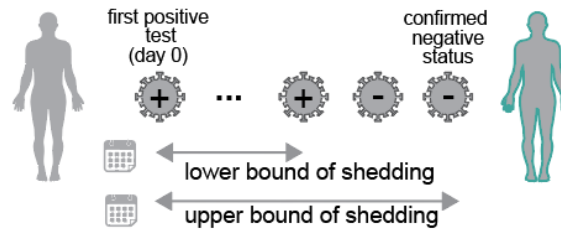
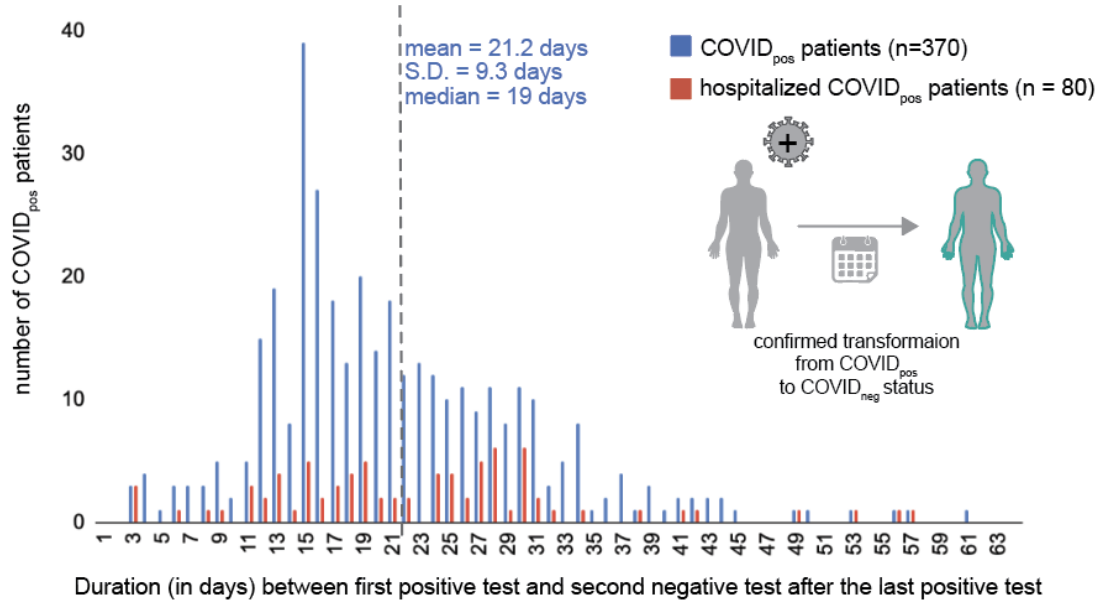


Figure 1

a Longitudinal testing in COVID-19 patients based on nasopharyngeal SARS-CoV-2 PCR tests



b Distribution of upper bound of viral shedding duration post SARS-CoV-2 PCR-based diagnosis



c Distribution of lower bound of viral shedding duration post SARS-CoV-2 PCR-based diagnosis

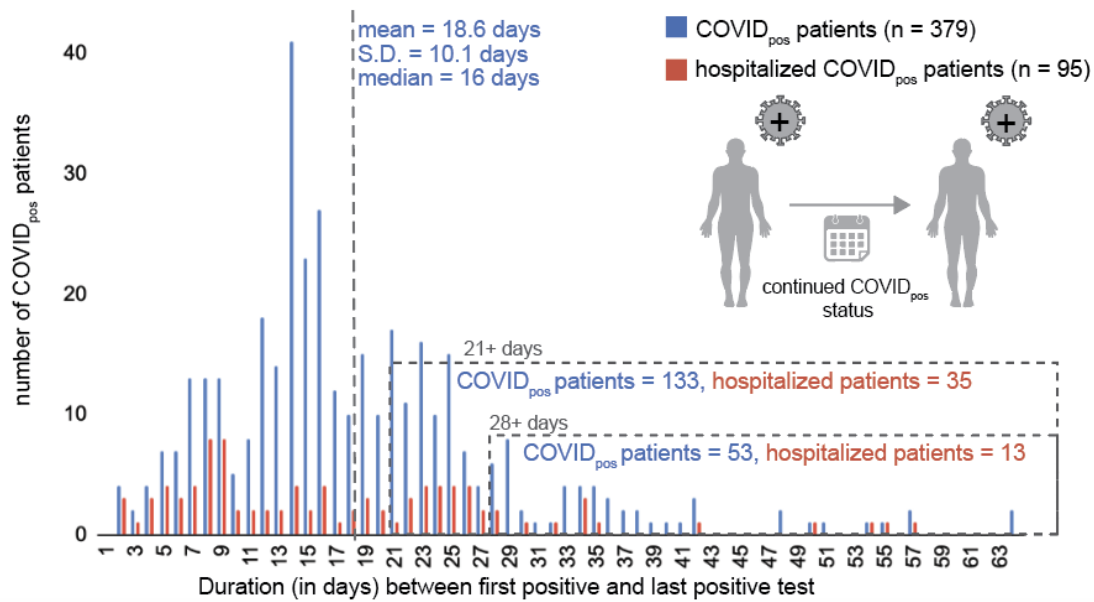
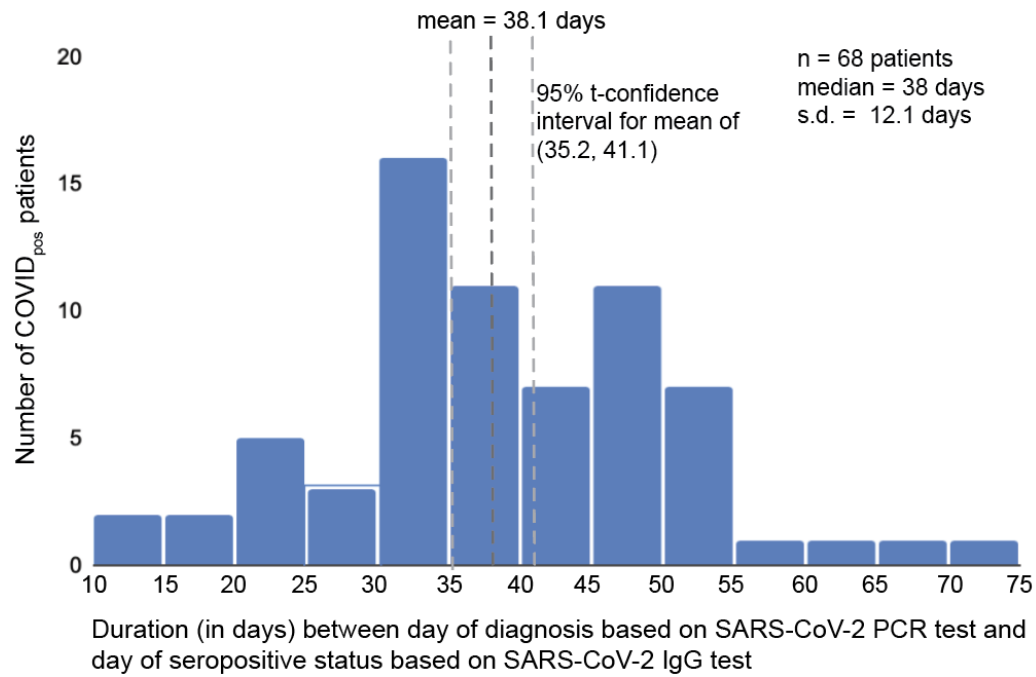


Figure 2

a Distribution of upper-bound of sero-positive transition duration based on SARS-CoV-2 IgG test



b Comparison of sero-positive status (based on antibody test) and COVID_{pos} status (based on PCR test)

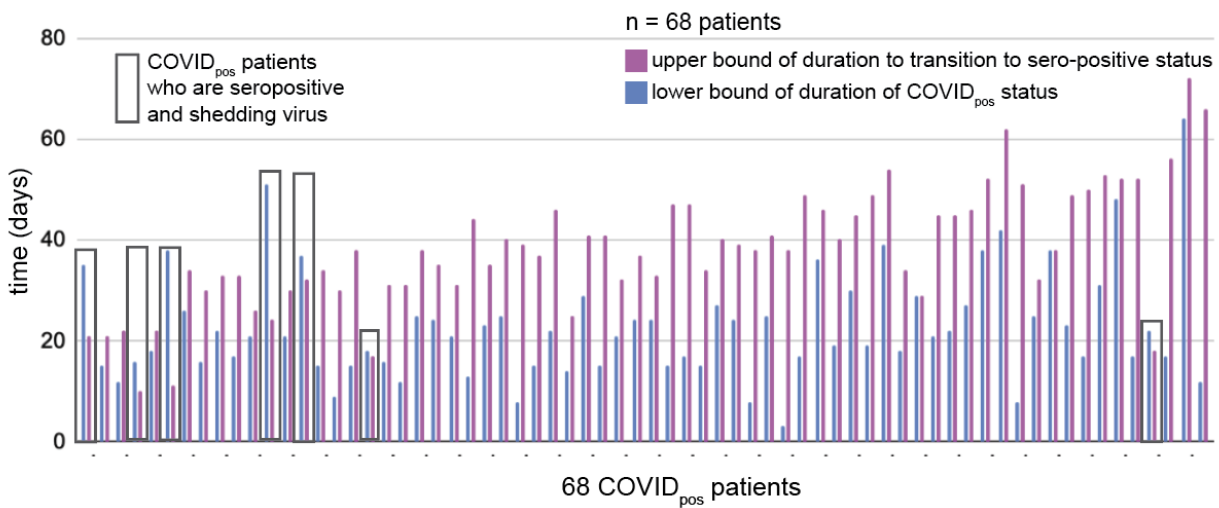


Figure 3

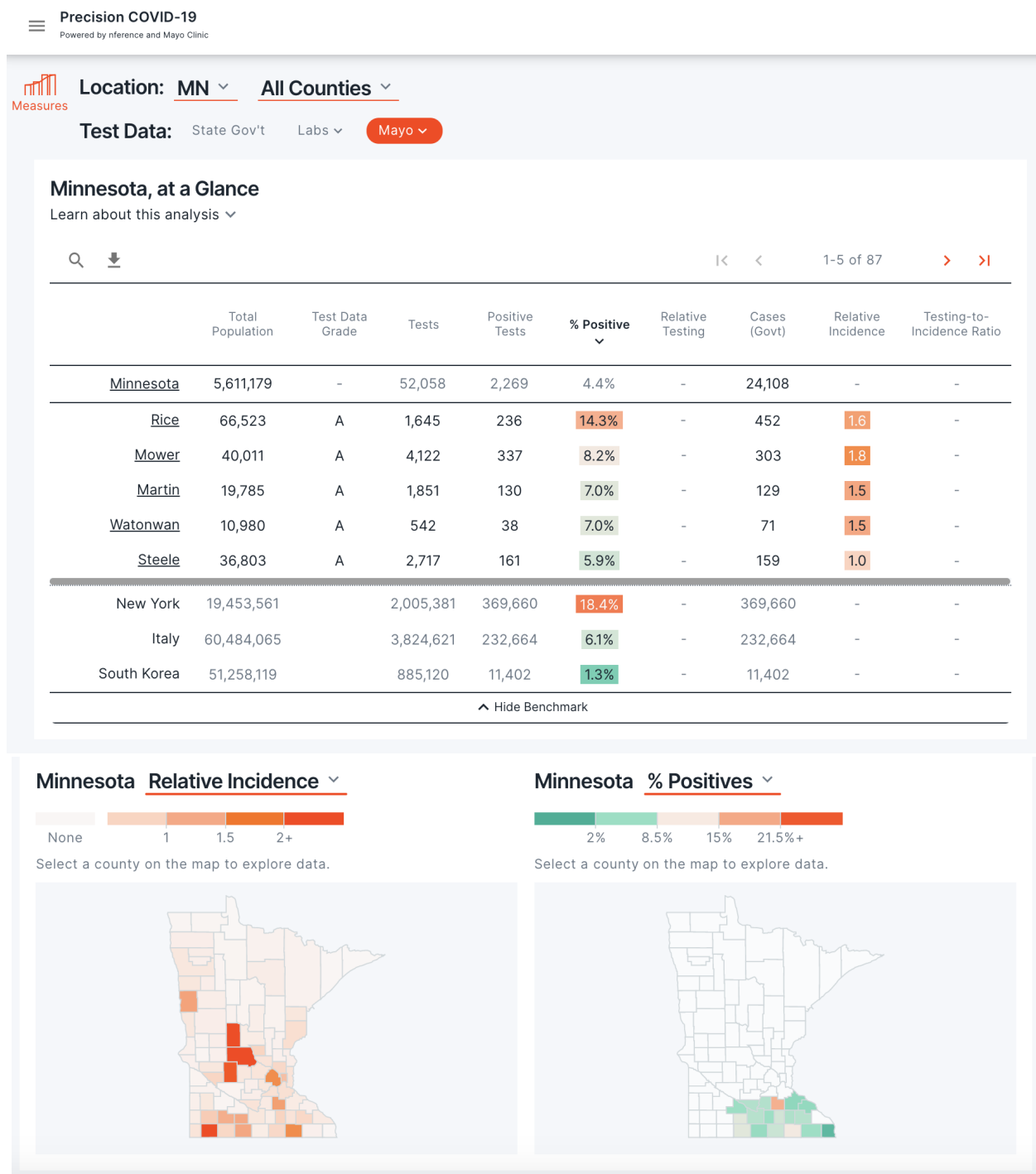


Figure 4