# Variation in SARS-CoV-2 molecular diagnostic performance by incidence of infection and symptomatology

Sanjat Kanjilal[1,2], Meghan Baker[1,2], Ann Woolley[2], Chanu Rhee[1,2], Athena Petrides[3], Junghyun Kim[2], Rajesh Patel[4], Rebecca Zaffini[3], Michael Klompas[1,2], Manfred Brigl[3]

[1]Department of Population Medicine, Harvard Pilgrim Healthcare Institute & Harvard Medical School, [2]Division of Infectious Diseases, Brigham & Women's Hospital, Boston, MA, [3]Department of Pathology, Brigham & Women's Hospital, Boston, MA, [4]Department of Medicine, Brigham & Women's Hospital, Boston, MA

## Introduction

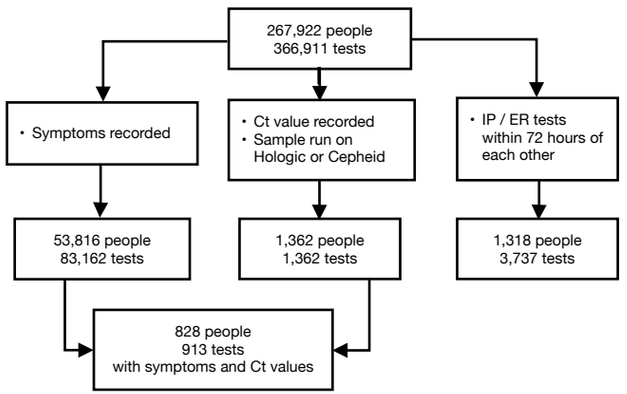
The reported test characteristics for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) PCR assays are derived from validation studies that are artificially enriched with samples containing high levels of virus, in order to meet regulatory standards. However, these data may be misleading when applied to patient populations where the distribution of viral burden differs significantly from validation studies.

We hypothesized that two factors were driving a decrease in the mean level of SARS-CoV-2 RNA in a population over time. First, as incidence of infection declines, the mean viral load of a patient is also expected to decline as people tend to be tested later in their disease course. Second, testing algorithms have expanded to include an increasing proportion of asymptomatic people, who have a lower mean viral load relative to symptomatic people. The combination of these trends can lead to significant deviations in test performance from the manufacturer's validation data. We tested our hypothesis using a retrospective analysis of testing data from a large healthcare system based in Massachusetts.

Our findings highlight the importance of periodic recalibration of test performance and re-evaluation of clinical decision pathways based on the characteristics of the patient population being tested and the local incidence of disease.

## Methods

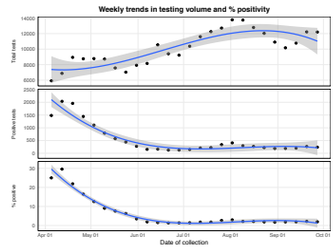
We performed a retrospective review of SARS-CoV-2 PCR test results obtained between 04/05/2020 and 10/03/2020 using 11 different PCR assays across the Mass General Brigham Healthcare System, located in Boston, MA.



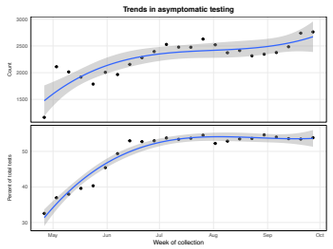

Calculation of sensitivity was performed for inpatients and ER patients with >1 test within a 72 hours period. A test was defined as a false negative if it was followed by a positive test within a 72 hours period. A test was considered a false positive if the Ct value was > 35, based on virology and epidemiologic data suggesting virus has not been transmitted when present at this low of a level. Prevalence was assumed to equal the percent positivity for a given week of data. Both analyses restricted to a person's 1st test.

## Trends in volume and rates of positivity

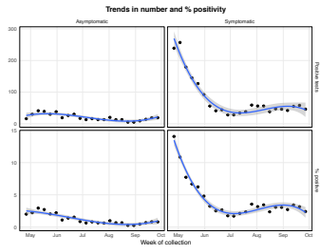
Testing capacity has increased even while rates of positivity of declined




The proportion of people being tested who are asymptomatic has increased to 50%

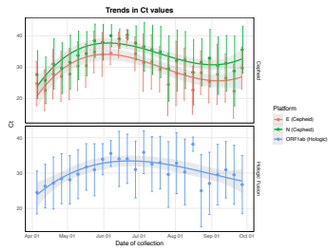



Rates of positivity in asymptomatic people are much lower than in symptomatics

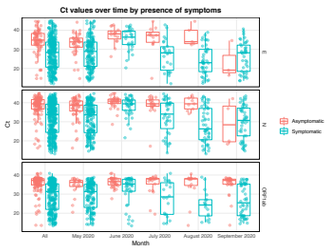



## Changes in Ct values over time and by patient populations

Mean Ct value increased as incidence declined and is now gradually decreasing as society re-opens



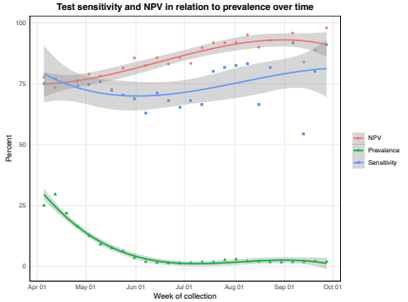

Ct values higher in asymptomatic than symptomatic people, but with overlap



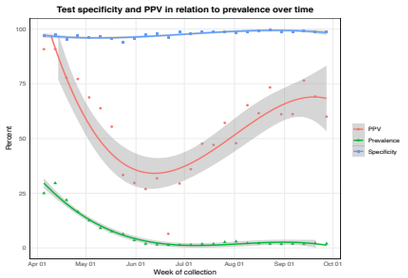

Likelihood of 1st test with Ct > 35 higher in asymptomatic people and closely track PPV for all people


## Test performance

Sensitivity varies by as much as 10%, while NPV benefits from low prevalence




Test specificity is relatively invariant but PPV heavily influenced by swings in prevalence




## Conclusions

We performed a large retrospective analysis from a regional health system in the New England area using detailed qualitative testing data combined with symptomatology and Ct values. Our results indicate that SARS-CoV-2 PCR test performance is highly dynamic and depends not only the local incidence of disease, but also the mean viral load of tested patients.

Hospital laboratory, infection control and operations leadership should acknowledge that the performance of SARS-CoV-2 assays are fluid and that test interpretation and algorithms require adjustment as local conditions change.

## Limitations

- Single center study in one region of the United States.
- Symptom and Ct value data limited to a subset of the tested population.
- Small numbers of people who are asymptomatic and tested positive with a recorded Ct value
- No gold standard assay for sensitivity or specificity

## Acknowledgments

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