

Letters

RESEARCH LETTER

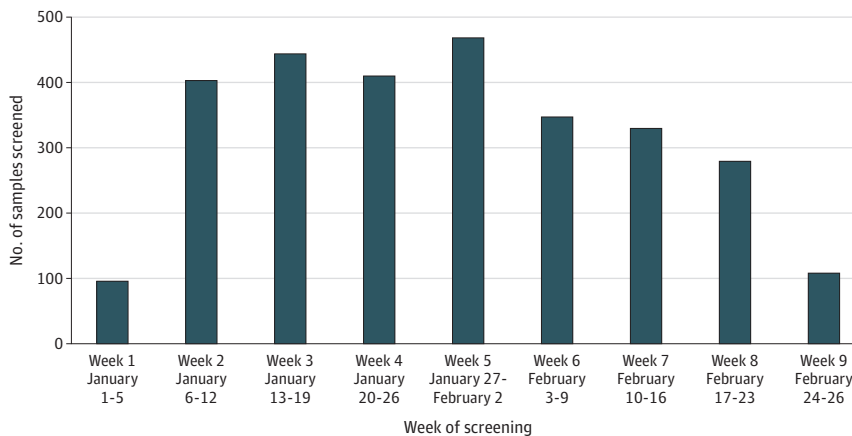
Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2

The coronavirus disease 2019 (COVID-19) pandemic has revealed the global importance of robust diagnostic testing to differentiate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from other routine respiratory infections and guide appropriate clinical management. Given the limited testing capacity available in the United States early in the pandemic, individuals with a clinical syndrome consistent with COVID-19, but without travel or exposure history, were not tested.¹ Therefore, it remains uncertain whether there may have been community circulation of SARS-CoV-2 prior to the identification of individuals with positive results through standard public health surveillance. Sample pooling, a strat-

egy used for community monitoring of other infectious diseases such as trachoma, has not, to our knowledge, been deployed for the early comprehensive screening of SARS-CoV-2 in the United States.²

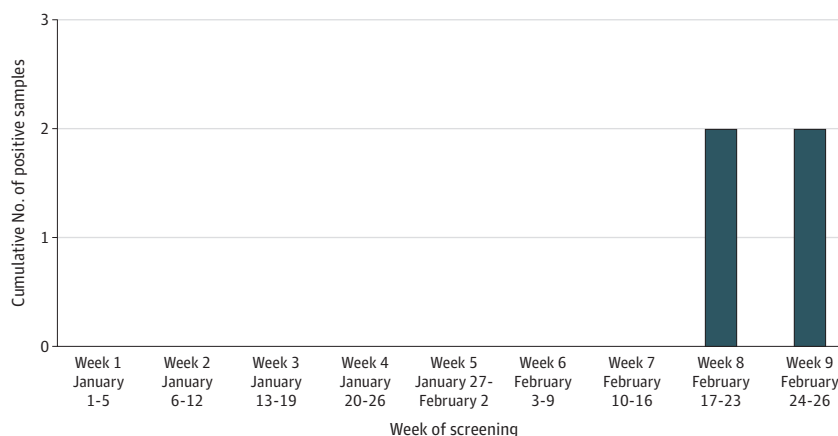
Methods | The Stanford Health Care Clinical Virology Laboratory serves adult and pediatric tertiary care hospitals and affiliated primary care and specialty clinics in the San Francisco Bay Area in California. We performed a retrospective study that evaluated all nasopharyngeal and bronchoalveolar lavage samples collected between January 1, 2020, and February 26, 2020, from inpatients and outpatients who had negative results by routine respiratory virus testing (respiratory pathogen or respiratory viral panels [GenMark Diagnostics] or Xpert Xpress Flu/RSV [Cepheid]) and had not been tested for SARS-CoV-2. After February 26, 2020, clinical

Figure 1. Number of Samples Screened for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)



Testing was performed by pooled sample screening at the Stanford Health Care Clinical Virology Laboratory over a 9-week period (January 1, 2020-February 26, 2020). Each pool included 9 to 10 individual samples that tested negative for other respiratory viruses. The number of SARS-CoV-2 samples, listed for weeks 1 through 9, were 96, 404, 444, 410, 469, 347, 330, 280, and 108. A total of 292 pools composed of 2888 individual samples were screened.

Figure 2. Cumulative Number of Positive Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Samples



Two positive samples were identified through pooled screening during week 8. Week 9 indicates the cumulative number for the screening period. See Figure 1 caption for testing details.

testing for SARS-CoV-2 on individual samples was begun, as recommended by institutional policy. Nine or 10 individual samples were pooled, and screening was performed using reverse transcriptase-polymerase chain reaction targeting the envelope (*E*) gene.³ Positive pools were deconvoluted and individual samples tested for both *E* and the RNA-dependent RNA polymerase (*RdRp*) gene for confirmation.³ This study was approved by the Stanford institutional review board. Given the deidentified nature of testing, individual patient consent was not required, as determined by the institutional review board, for this study.

Results | A total of 292 pools were screened, corresponding with 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples (Figure 1). The confirmed positivity rate for SARS-CoV-2 was 0.07% (2/2888) (Figure 2). The positive results were from nasopharyngeal samples collected on February 21, 2020, and on February 23, 2020. The 2 positive samples showed detection of *E* and *RdRp*. Sanger sequencing revealed 100% identity with the SARS-CoV-2 *E* gene. Only 1 pool showed a positive *E* signal that was not reproducible with testing of the individual samples of that pool.

Discussion | Results from this screening strategy support that the burden of disease in the San Francisco Bay Area early in the pandemic was low; less than 1% of all symptomatic individuals with negative routine testing had SARS-CoV-2 infection. The timing of the positive pools overlapped with the first 3 individuals with positive results reported from Santa Clara County, tested using criteria established by the Centers for Disease Control and Prevention.^{4,5} Thus, public health counts of individuals with SARS-CoV-2 infection indicated a reasonable estimate of overall disease burden among symptomatic individuals in this area.⁶ Nevertheless, the individuals identified with positive results via this screening strategy would not have met the existing testing criteria.

A pooled screening strategy was pursued to increase testing throughput, limit use of reagents, and increase overall testing efficiency at an expected slight loss of sensitivity. With only 1 false-positive reading, the strategy was specific. Due to the challenges of restricted access to diagnostic tests and kit supplies across the United States, early testing has largely been limited to symptomatic individuals fulfilling testing criteria.⁴ Although this approach facilitates rational use of resources, it may miss individuals in whom COVID-19 risk has not been identified.⁴ This study is limited in that it was performed in a single laboratory in a restricted geo-

graphical area; additional data are thus required to validate this approach on a larger scale. Furthermore, this screening strategy does not obviate the need for individual diagnostic testing, particularly as community transmission intensifies.

Strategies such as pooled screening may facilitate detection of early community transmission of SARS-CoV-2 and enable timely implementation of appropriate infection control measures to reduce spread.

Catherine A. Hogan, MD, MSc
Malaya K. Sahoo, PhD
Benjamin A. Pinsky, MD, PhD

Author Affiliations: Department of Pathology, Stanford University School of Medicine, Stanford, California.

Corresponding Author: Benjamin A. Pinsky, MD, PhD, Department of Pathology, Stanford University School of Medicine, 3375 Hillview, Room 2913, Palo Alto, CA 94304 (bpinsky@stanford.edu).

Published Online: April 6, 2020. doi:10.1001/jama.2020.5445

Author Contributions: Drs Hogan and Pinsky had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: All authors.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Hogan, Pinsky.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Hogan, Sahoo.

Administrative, technical, or material support: Pinsky.

Supervision: Pinsky.

Conflict of Interest Disclosures: None reported.

Additional Contributions: We thank Chunhong Huang, PhD, Department of Pathology, Stanford University, for her contribution in generating the data for this study, without compensation.

1. Sharfstein JM, Becker SJ, Mello MM. Diagnostic testing for the novel coronavirus. *JAMA*. Published online March 9, 2020. doi:10.1001/jama.2020.3864
2. Ray KJ, Zhou Z, Cevallos V, et al. Estimating community prevalence of ocular Chlamydia trachomatis infection using pooled polymerase chain reaction testing. *Ophthalmic Epidemiol*. 2014;21(2):86-91. doi:10.3109/09286586.2014.884600
3. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):1-8. doi:10.2807/1560-7917.ES.2020.25.3.2000045
4. Centers for Disease Control and Prevention. Evaluating and testing persons for coronavirus disease 2019 (COVID-19). Updated March 24, 2020. Accessed March 25 2020. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-criteria.html>
5. Santa Clara County Public Health. Healthy Santa Clara County: novel coronavirus (COVID-19) data dashboard. Accessed March 25, 2020. <https://www.sccgov.org/sites/phd/DiseaseInformation/novel-coronavirus/Pages/dashboard.aspx>
6. Lipsitch M, Swerdlow DL, Finelli L. Defining the epidemiology of COVID-19—studies needed. *N Engl J Med*. 2020;382(13):1194-1196. Published online March 26, 2020. doi:10.1056/NEJMp2002125