Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Screening With Specimen Pools: Time to Swim, or Too Deep for Comfort?

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(See the Brief Report by Li et al on pages 318–20.)

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The outbreak and global spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to challenge the way physicians, laboratories, and public health officials diagnose and track cases. Testing was initially relegated to reference laboratories and academic medical centers with the expertise to rapidly design and validate laboratory-developed diagnostic assays to detect a novel virus. These laboratories quickly became overburdened, resulting in turnaround times beyond what is clinically actionable. The availability of commercial SARS-CoV-2 diagnostics decentralized coronavirus disease 2019 (COVID-19) testing algorithms, resulting in increased testing capacity on a national level. However, despite the Herculean efforts of physicians, public health officials, and clinical laboratory personnel, significant challenges and case backlogs remain.

As we learn more about COVID-19 transmission, countries worldwide have tried to curtail disease spread via containment efforts, including quarantines, closure of municipal spaces, and compulsory social distancing. Due to limited availability, testing initially focused on the identification of symptomatic individuals. As parts of the world reported decreased caseloads, the focus has shifted to include screening of asymptomatic individuals in community settings in order to prevent future outbreaks. Similar to routine diagnostics, screening tests need to be both analytically and clinically sensitive and easy to perform. Screening tests also carry an inherent requirement to be reasonably inexpensive and able to identify large numbers of affected yet asymptomatic patients. The optimal specimen type, diagnostic technology, sampling format, and screening strategy for SARS-CoV-2 remain unknown; this uncertainty is compounded by unstable testing supply chains.

We read with interest the study appearing in this issue by Li et al, who utilized a pooled sample strategy and a point-of-care (POC) reverse transcriptase-polymerase chain reaction (RT-PCR) assay for screening asymptomatic airline passengers arriving from areas of high SARS-CoV-2 prevalence. The study demonstrates sample pooling as a mechanism for both cost reduction and resource conservation, using a commercial molecular platform with a rapid turnaround time and strong analytical sensitivity. While we view the results of this work as an overwhelmingly positive step forward, it is important to consider the context in which this strategy was deployed and the impact of the location and population on the results.

Sample pooling is a method of increasing the throughput of diagnostic assays. In this approach, small volumes of samples from multiple patients are combined into a single test, resulting in substantial reagent savings. If the pooled sample returns a negative result, all patients with specimens comprising that pool are considered not to be infected. A positive result requires that the pool be deconvoluted, or split, into its representative parts, and each component sample is tested individually to identify the infected patient(s). Sample pooling was evaluated for tracking SARS-CoV-2 in a community setting early in the US outbreak [1], and has been used historically as a public health tool for the detection of other viral agents, including human immunodeficiency virus. At the time of this writing, 2 reference laboratories in the United States (Quest Diagnostics and LabCorp) have received emergency use authorizations from the US Food and Drug Administration to use pooled specimens for SARS-CoV-2 detection [2].
In this work, pooling was performed in a 10:1 ratio, meaning 10 patient specimens were combined and tested using a single SARS-CoV-2 assay. The number of SARS-CoV-2 specimens per pool varies in the limited number of investigations reported to date [1, 3–5]. While the potential financial and reagent savings are obvious, careful and rigorous investigation is necessary to assure the pooling of specimens does not impact the analytical sensitivity of the assay [3, 4]. This is particularly important in a screening test used for asymptomatic individuals, who may not have high levels of detectable viral material present in their clinical specimens. Indeed, in this work, RT-PCR cycle threshold (Ct) values of nasopharyngeal swabs from the 2 positive, asymptomatic patients were 40.6 and 41.6 when tested alone, and 43.8 and 43.9 when tested in the context of a 10:1 pool. These values are at the extreme end of the analytical sensitivity of this assay, highlighting the need for careful experimental design.

Li et al pooled specimens from patients traveling from high-risk areas in mainland China to Hainan Island as part of a SARS-CoV-2 disembarkation screening process at Sanya Airport. A particularly remarkable aspect of this work is that all testing (including positive pool deconvolution) was performed on site at the airport. Most investigations of SARS-CoV-2 sample pooling utilize laboratory-developed assays that must be performed in approved biosafety level 2 settings, require specialized instrumentation, require trained technical staff, and are not portable. By taking advantage of the ease of operation of the Cepheid platform, sample analyses were able to be performed in real time, allowing for rapid intervention.

This approach may be feasible for an island featuring limited points of entry and a more isolated population. It is, however, challenging to extrapolate this study to an effective screening protocol for use in schools, workplaces, and sporting events in geographical areas with more porous borders. Additionally, for sample pooling to be an effective strategy, the test population must have a low prevalence rate. The total number of COVID-19 cases in Hainan at the time of writing was 170. This number is significantly lower than the daily number of cases reported by some US cities, highlighting the utility of this approach in a low-prevalence setting. Locales with higher SARS-CoV-2 prevalences will require more frequent deconvolution of positive patient pools, thus chipping away at or negating the benefits of a pooling approach.

There are several practical points to resolve before a test-based screening policy can be deployed. Beyond the obvious financial concerns, a major issue is the testing turnaround time. To date, most of the available “rapid” POC platforms repeatedly demonstrate lower levels of analytical sensitivity. In the United States, this means the molecular testing would need to be performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, which could again lead to the overburdening of clinical laboratories; a notable exception is the Cepheid platform for SARS-CoV-2, which is CLIA-waived and has good analytical sensitivity, hence its use in this study. Moreover, the simple reality of specimen collection, transportation, testing, and reporting further prolongs turnaround times in settings without access to an on-site laboratory (ie, schools, business, etc.). This might imply that patients need to be tested the day before results are to be reviewed; in other words, get swabbed on Sunday to go to work on Monday.

This leads to a second and possibly more important question: what does a negative or positive result mean? At our institution, we are aware of patients who underwent preprocedure SARS-CoV2 screening utilizing the same assay deployed in this work, only to be diagnosed with active, symptomatic COVID-19 within 5 days of testing. As knowledge about COVID-19 increases, it appears that the predictive value of a negative test in an asymptomatic person depends on the timing of sample collection in the context of the patient’s disease course [6]. Therefore, if an institution utilizes a weekly screening protocol, and screened individuals are still in their incubation period, it is possible they could test negative on Monday, become infectious by Wednesday, but not show symptoms until Friday. If people misinterpret negative test results as justification to relax their nonpharmacologic interventions (mask wearing), especially at traditionally social times like lunch and coffee breaks, the magnitude of disease spread could be significant.

Conversely, in the absence of robust data concerning viral shedding, infectivity, and correlation to RT-PCR Ct values, testing of asymptomatic patients using an assay with high analytical sensitivity begs the question as to whether such patients are truly contagious. Of the 2 positive patients detected in this work, both exhibited immunoglobulin G seropositivity and had nasopharyngeal samples with elevated Ct values; 1 had an exposure history spanning up to 6 months before testing. It is not possible to know whether these patients are truly infectious without additional studies. Thus, until we learn more about the biology and transmission kinetics of SARS-CoV-2, it is difficult to ascertain the clinical significance of such results.

It might be reasonable to deploy a test-based screening strategy in a setting with controlled points of entry, a rapid turnaround time, effective means of contacting those who are tested, a low disease prevalence, and high compliance with masking. As these prerequisites are unlikely to be met in the contiguous United States, we either need to reconsider the utility of test-based screening or find faster, cheaper, and more sensitive POC tests. Until this happens, pooled testing is an option to reduce costs and speed results.
Note
Potential conflicts of interest. The authors: No reported conflicts of interest. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References
1. Hogan CA, Sahoo MK, Pinsky BA. Sample pooling as a strategy to detect community transmission of SARS-CoV-2. JAMA 2020; 323:1967–9.
2. United States Food and Drug Administration. In vitro diagnostics EUAs. 2020. Available at: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas. Accessed 27 July 2020.
3. Lohse S, Pfühl T, Berkó-Göttel B, et al. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people [manuscript published online ahead of print 28 April 2020], Lancet Infect Dia. 2020; S1473-3099(20)30362-5. doi:10.1016/s1473-3099(20)30362-5
4. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. Assessment of specimen pooling to conserve SARS-CoV-2 testing resources. Am J Clin Pathol 2020; 153:715–8.
5. United States Food and Drug Administration. Coronavirus (COVID-19) update: FDA issues first emergency authorization for sample pooling in diagnostic testing. 2020. Available at: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-first-emergency-authorization-sample-pooling-diagnostic. Accessed 27 July 2020.
6. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med 2020; 173:262–7. doi:10.7326/M20-1495.