Optimizing SARS-CoV-2 Pooled Testing Strategies Through Differentiated Pooling for Distinct Groups

Lindsey M. Filiatreau*, Paul N. Zivich, Jessie K. Edwards, Grace E. Mulholland, Ryan Max, and Daniel Westreich

* Correspondence to Dr. Lindsey Filiatreau, Department of Psychiatry, Washington University in St. Louis, 660 S. Euclid, Street Louis, MO 63110 (e-mail: flindsey@wustl.edu, lfiliatreau@gmail.com).

Initially submitted March 25, 2021; accepted for publication October 6, 2022.

Pooled testing has been successfully used to expand SARS-CoV-2 testing, especially in settings requiring high volumes of screening of lower-risk individuals, but efficiency of pooling declines as prevalence rises. We propose a differentiated pooling strategy that independently optimizes pool sizes for distinct groups with different probabilities of infection to further improve the efficiency of pooled testing. We compared the efficiency (results obtained per test kit used) of the differentiated strategy with a traditional pooling strategy in which all samples are processed using uniform pool sizes under a range of scenarios. For most scenarios, differentiated pooling is more efficient than traditional pooling. In scenarios examined here, an improvement in efficiency of up to 3.94 results per test kit could be obtained through differentiated versus traditional pooling, with more likely scenarios resulting in 0.12 to 0.61 additional results per kit. Under circumstances similar to those observed in a university setting, implementation of our strategy could result in an improvement in efficiency between 0.03 to 3.21 results per test kit. Our results can help identify settings, such as universities and workplaces, where differentiated pooling can conserve critical testing resources.

COVID-19; pooled testing; SARS-CoV-2; testing efficiency

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UNC, University of North Carolina.

Coronavirus disease 2019 (COVID-19) was the third leading cause of death in the United States in 2020 (1) and remains a leading cause of death globally (2, 3). Public health professionals have emphasized that increased testing is a key strategy to minimize transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and decrease subsequent COVID-19 mortality (4–9). In settings such as universities and workplaces, wide-scale testing of asymptomatic individuals is gaining traction as an infection control mechanism, with many administrators implementing mandated testing policies, generally in lieu of proof of vaccination (10–12). For example, unvaccinated students enrolled in courses at the University of North Carolina (UNC) at Chapel Hill in Fall 2021 were required to test twice weekly and unvaccinated employees were required to test once weekly (13). Testing for individuals who were either symptomatic or were known close contacts of an individual testing SARS-CoV-2 positive was recommended regardless of vaccination status (13). However, as new SARS-CoV-2 variants emerge and sweep the globe, facility- and system-level barriers such as supply chain bottlenecks and laboratory throughput capacity continue to impede efficient scale-up of testing efforts across a number of settings (6, 14–18).

Pooled testing, also called group testing, is an established and effective testing strategy that can conserve testing resources, save personnel time, minimize turnaround time from testing to receipt of results, and improve testing efficiency (i.e., increase the number of results obtained or individuals screened per test kit used) (10, 19–32). Pooled testing is a process by which multiple specimens are combined (or "pooled") and the pooled samples are screened for the agent of interest (in this case, SARS-CoV-2). Pools that screen positive are then tested again in subpools or

as individual samples (33). In a recent article on pooled testing as a means for optimizing the efficiency of SARS-CoV-2 testing, the authors demonstrated that for a given number of test kits, testing programs using pooled testing could screen between 2 and 20 times as many specimens when compared with programs using individual-specimen testing (19). However, in high-prevalence settings where a large proportion of individual samples would be SARS-CoV-2-positive, the efficiency gains of pooled testing are reduced or eliminated, and improvements in turnaround time obtained through pooling are lost because a large proportion of pools would screen positive, necessitating further subpool or individual-specimen testing (34).

We propose a differentiated pooled testing strategy, similar to the Informative Dorfman Screening strategy previously proposed by McMahan et al. for use in chlamydia and gonorrhea testing (35), to improve efficiency gains of pooled SARS-CoV-2 testing across a range of settings, including high-prevalence settings and lower-prevalence settings where identifiable subgroups of the testing population are at substantially higher risk of infection. In this paper, we explore the improvement in efficiency, expressed as the number of additional test results that can be obtained per test kit used, through implementation of our considered strategy versus a traditional 2-stage hierarchal (i.e., "pooling") testing strategy where samples are processed in uniform pool sizes (27).

METHODS

Overall strategy

In a traditional 2-stage pooling strategy, samples are processed in uniform pool sizes, and the calculation of the optimal pool size is based on the prevalence of SARS-CoV-2 in the overall population being tested. In a differentiated pooled testing strategy (i.e., "differentiated pooling"), pool sizes are independently optimized for 2 or more distinct groups with different test-positive probabilities. Here we will consider 2 easily distinguishable groups with characteristics shown to be reliable predictors of infection: 1) a higher-risk group in which individuals have known or probable exposure to SARS-CoV-2 or self-report symptoms consistent with COVID-19 (i.e., "symptomatic or exposed individuals"); and 2) a lower-risk group in which individuals are asymptomatic and have no known or probable exposure to SARS-CoV-2 (i.e., "asymptomatic and unexposed individuals") (36). Optimal pool sizes are determined separately for the 2 groups based on a range of SARS-CoV-2 prevalences in each group, and pools screening positive are subsequently tested as individual samples.

Calculating efficiency

We consider the average number of individual results obtained per test kit used as a measure of efficiency. Computing testing strategy efficiency requires assumptions regarding the diagnostic test performance, specifically the assay sensitivity (S_e) and specificity (S_p), and local viral dynamics, specifically the prevalence of SARS-CoV-2 (P(D)) in the population of interest. Given the loss in diagnostic sensitivity that can result from pooling specimens together (as positive samples are diluted with negative samples) (34, 37), a maximum allowable dilution, or the proportion of diagnostic sensitivity one is willing to lose through pooling, should be established. This determines the maximum allowable pool size, or maximum pool size that preserves the diagnostic sensitivity established by the maximum allowable dilution (19). Additional details regarding dilution effects and estimation of the maximum allowable dilution and pool size are provided in Web Appendix 1 (available at https://doi.org/10.1093/aje/kwac178).

With these input parameters defined, the efficiency of a particular testing strategy can be calculated using the methods and viral dynamics model originally developed by Pilcher et al. (19). We have adapted these methods for our specific test strategy approach and outlined the details in Web Appendix 1.

Estimating efficiency of a differentiated pooled testing strategy (ψ)

In calculating the efficiency, or number of results obtained per test kit used, in our differentiated pooling strategy (ψ), we first estimated the optimal pool size for each of our 2 groups (symptomatic or exposed individuals; asymptomatic and unexposed individuals) separately. To estimate these pool sizes, we used the prevalence of infection among symptomatic or exposed individuals (P($D|G = 1$)) and the prevalence of infection among asymptomatic and unexposed individuals (P($D|G = 0$)). We considered 2 sets of assay performance scenarios: 1) a conservative diagnostic assay S_e of 0.75 and S_p of 0.95 among symptomatic or exposed individuals and S_e of 0.50 and S_p of 0.95 among asymptomatic and unexposed individuals; and 2) a higher S_e of 0.85 and S_p of 0.99 among symptomatic or exposed individuals and S_e of 0.60 and S_p of 0.99 among asymptomatic and unexposed individuals (38–40). We allowed the diagnostic assay sensitivity and specificity to differ between the 2 groups as literature suggests that assay performance is diminished in cases of mild infection (41–44). In each scenario, we set the maximum allowable dilution at 0.20 to ensure the reduction in analytical sensitivity of pooled SARS-CoV-2 testing was less than 20% relative to individual-specimen testing. Using the viral dynamics model proposed by Pilcher et al. (19) and further outlined in Web Appendix 1, this corresponds to a maximum allowable pool size of 25 in all scenarios.

After determining the optimal pool size for each group, we used the adapted viral dynamics model outlined in Web Appendix 1 to calculate the efficiency, or number of results obtained per test kit used, in each group, separately. Finally, we calculated ψ by taking a weighted average of the efficiency in symptomatic or exposed individuals ($\varphi_{G=1}$) and the efficiency in asymptomatic and unexposed individuals ($\varphi_{G=0}$) using the formula:

$$\psi = (\varphi_{G=1} \times P(G = 1)) + (\varphi_{G=0} \times P(G = 0)),$$

where P($G = 1$) is the proportion of individuals in the population being tested who self-report symptoms or known

or probable exposure to SARS-CoV-2 and $P(G = 0)$ is the proportion of individuals in the population being tested who self-report no known symptoms or exposure to SARS-CoV-2.

Estimating efficiency of a traditional pooled testing strategy (φ)

In estimating the efficiency of a traditional pooling strategy in the overall testing population (φ), we first estimated the optimal pool size using the prevalence of infection in the overall population of individuals being tested, $P(D)$, and the assay sensitivity and specificity in the overall population of individuals being tested. $P(D)$ was calculated via using $P(D|G = 1)$, $P(D|G = 0)$, $P(G = 1)$, and $P(G = 0)$:

$$P(D) = (P(D|G = 1) \times P(G = 1)) + (P(D|G = 0) \times (P(G = 0))$$

Assay sensitivity and specificity in the population being tested were determined in a similar manner using weighted averages. After determining the optimal pool size and assay sensitivity and specificity in the testing population overall, we then used the adapted viral dynamics model outlined in Web Appendix 1 to estimate φ.

Estimating change in efficiency (Δ) from implementation of differentiated versus traditional pooling

To demonstrate the improvement in efficiency (Δ) associated with differentiated versus traditional pooling in the overall population of individuals being tested, we compared the results obtained per test kit used in a differentiated pooling strategy (ψ) with the results obtained per test kit used in a traditional pooling strategy (φ) via $\Delta = \psi - \varphi$. Here, the difference in estimates, Δ, is the total improvement in efficiency, or number of additional individual test results that can be obtained per test kit used through implementation of differentiated compared with traditional pooling.

First, we examined Δ for scenarios similar to those observed in the Carolina Together Testing Program at UNC-Chapel Hill between August 18, 2021, and August 31, 2021, during the first 2 weeks of classes. During this period, 12,629 individuals were tested, 91% of whom self-reported to be asymptomatic and unexposed, and were tested as a part of the requirement for unvaccinated students and faculty (13). The percent testing positive was 1.9 among asymptomatic

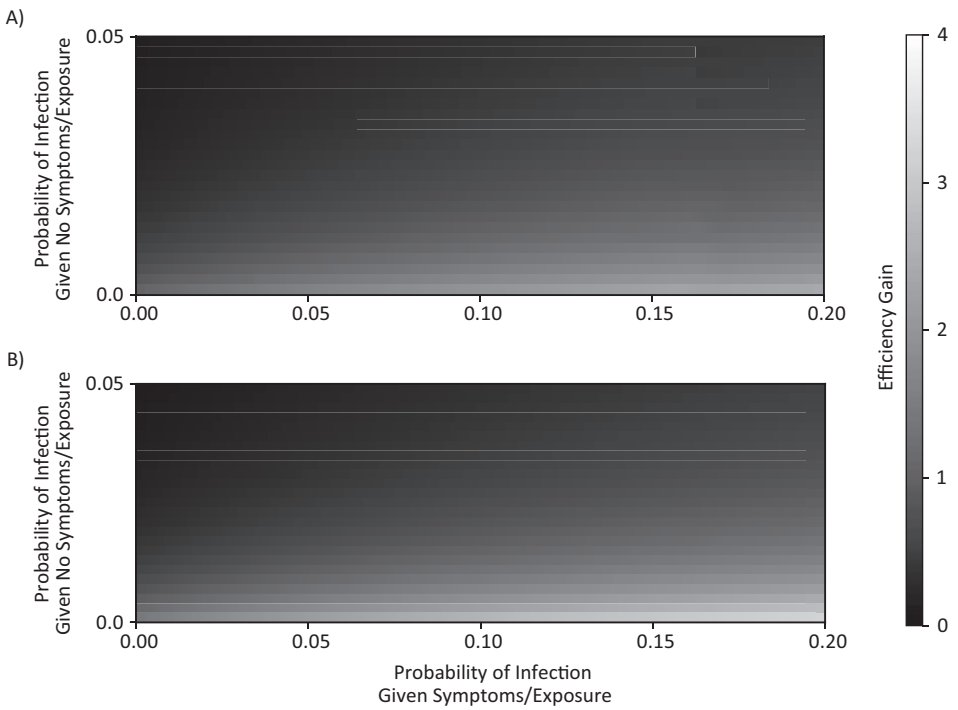

Figure 1. Improvement in efficiency, or additional results obtained per test kit used (Δ) through implementation of a differentiated versus traditional pooling strategy, in settings similar to those observed at the University of North Carolina at Chapel Hill, August 2021. Settings with a probability of infection given symptomatic or exposed ($P(D|G = 1)$) ranging from 0% to 20%, a probability of infection given asymptomatic and unexposed ($P(D|G = 0)$) ranging from 0% to 5%, and a probability of being symptomatic or exposed in the population being tested ($P(G = 1)$) of 10% were explored. A) Assay sensitivity and specificity were set to 0.75 and 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals. B) Assay sensitivity and specificity were set to 0.85 and 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals.

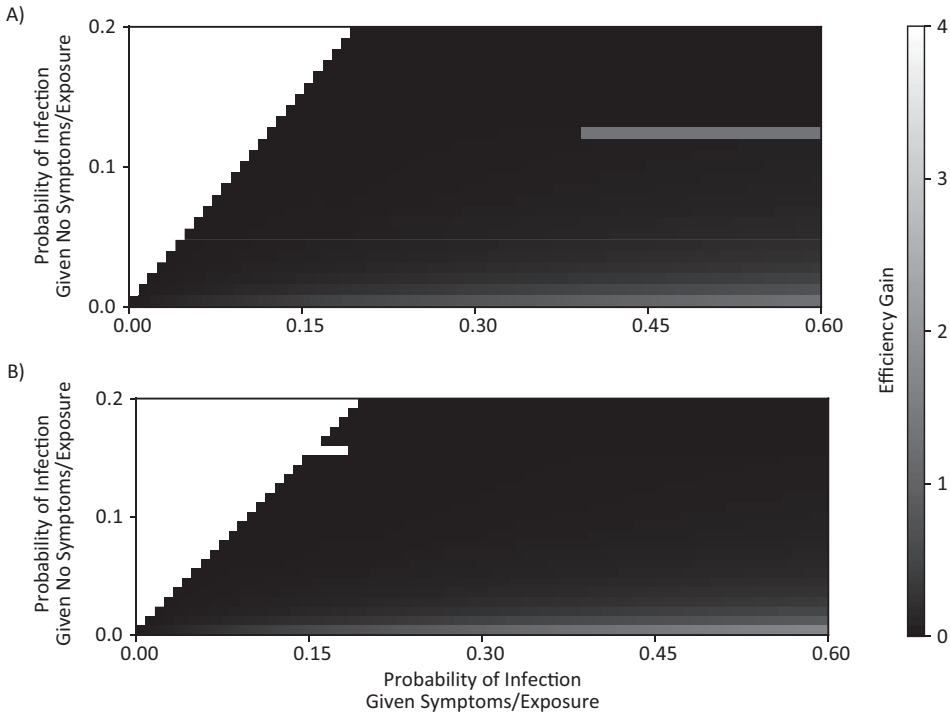

Figure 2. Improvement in efficiency, or additional results obtained per test kit used (Δ) through implementation of a differentiated versus traditional pooling strategy in settings with a probability of infection given symptomatic or exposed (P($D|G = 1$)) ranging from 0% to 60%, a probability of infection given asymptomatic and unexposed (P($D|G = 0$)) ranging from 0% to 20%, and a probability of being symptomatic or exposed in the population being tested (P($G = 1$)) of 1%. A) Assay sensitivity and specificity were set to 0.75 and 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals. B) Assay sensitivity and specificity were set to 0.85 and 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals. White areas represent scenarios in which P($D|G = 0$) is greater than P($D|G = 1$) or traditional pooling is more efficient than differentiated pooling (i.e., $\Delta < 0$).

and unexposed individuals and 6.2 among symptomatic or exposed individuals (13). To explore scenarios similar to the observed data, we allowed P($D|G = 1$) to range from 0.00 to 0.20 and P($D|G = 0$) to range from 0.00 to 0.05 at P($G = 1$) of 0.10. These estimates of Δ are presented visually in a heat map in which we highlight scenarios where differentiated pooling is equivalent to or more efficient than traditional pooling (i.e., $\Delta \geq 0$) and P($D|G = 0$) is less than P($D|G = 1$) (Figure 1; Web Figure 1). These scenarios are most plausible in settings where there is screening both for asymptomatic and unexposed individuals and for symptomatic or exposed individuals (e.g., universities, workplaces).

We then explored Δ under a range of scenarios for P($D|G = 0$), P($D|G = 1$), and P($G = 1$). Specifically, we estimated Δ for P($D|G = 1$) values ranging from 0.01 to 0.60 and P($D|G = 0$) values ranging from 0.00 to 0.20 at P($G = 1$) values of 0.01, 0.10, 0.50, and 0.75. Under this set of parameters, P(D) values ranged from <0.01% to 40%. Estimates of Δ in each of these scenarios are presented in an additional series of heat maps (Figures 2–5; Web Figures 2–5). We present estimates of the optimal pool size for select values in tables (Tables 1 and 2). A comprehensive range of scenarios (i.e., where P($D|G = 1$) values range from 0.00 to 0.99, P($D|G = 0$) values range from 0.00 to 0.50, and P($G = 1$) values range from 0.00 to 0.99) that could represent the circumstances of nearly all testing programs are presented in Web Videos 1 (conservative assay performance) and 2 (higher assay performance).

RESULTS

Under conditions similar to those observed at UNC-Chapel Hill during the first 2 weeks of classes, assuming a conservative assay sensitivity of 0.75 and specificity of 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals, the efficiency estimates for differentiated pooling (ψ) were typically higher than the efficiency estimates for traditional pooling (φ) (i.e., $\Delta > 0$) when P($D|G = 1$) exceeded P($D|G = 0$). Specifically, an improvement in efficiency of between 0.03 and 2.37 results per test kit could be obtained through implementation of differentiated versus traditional pooling (Figure 1A). Slightly higher estimates of Δ could be obtained when utilizing a higher-performing assay (Δ = 0.03 to 3.21; Figure 1B).

In a population where just 1% of individuals are symptomatic or exposed and P($D|G = 1$) exceeds P($D|G = 0$), ψ is, under most scenarios, similar to φ (i.e., $\Delta \sim 0$) (Figure 2). However, an improvement in efficiency of up

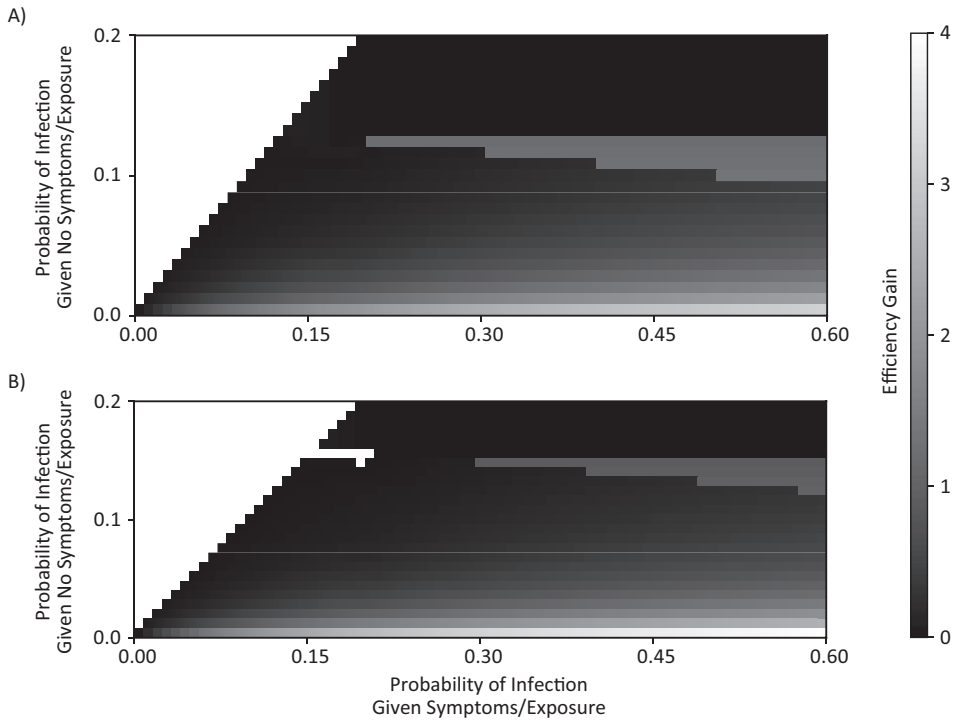

Figure 3. Improvement in efficiency, or additional results obtained per test kit used (Δ) through implementation of a differentiated versus traditional pooling strategy in settings with a probability of infection given symptomatic or exposed ($P(D|G = 1)$) ranging from 0% to 60%, a probability of infection given asymptomatic and unexposed ($P(D|G = 0)$) ranging from 0% to 20%, and a probability of being symptomatic or exposed in the population being tested ($P(G = 1)$) of 10%. A) Assay sensitivity and specificity were set to 0.75 and 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals. B) Assay sensitivity and specificity were set to 0.85 and 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals. White areas represent scenarios in which $P(D|G = 0)$ is greater than $P(D|G = 1)$ or traditional pooling is more efficient than differentiated pooling (i.e., $\Delta < 0$).

to 1.70 results per test kit (i.e., Δ up to 1.70) could be obtained (Figure 2). In a population where 10% of individuals are symptomatic or exposed, an improvement in efficiency of up to 3.97 results per test kit could be obtained through implementation of a differentiated versus traditional pooling strategy (Figure 3). In a population where 50% of individuals are symptomatic or exposed, an improvement in efficiency of up to 2.95 results per test kit could be obtained through implementation of a differentiated versus traditional pooling strategy (Figure 4). In a population where 75% of individuals are symptomatic or exposed, an improvement in efficiency of up to 1.48 results per test kit could be achieved (Figure 5). In these scenarios, the improvement in efficiency obtained through differentiated versus traditional pooling was most often between 0.12 and 0.61 results per test kit. There were some circumstances where traditional pooling was more efficient than differentiated pooling, with values of Δ between -1 and 0 (see white portion of figures; Table 1).

Results observed under improved assay sensitivity and specificities (i.e., $S_e = 0.85$ and $S_p = 0.99$ among symptomatic or exposed individuals and $S_e = 0.60$ and $S_p = 0.99$ among asymptomatic and unexposed individuals) were largely similar to those observed under more conservative assay sensitivity and specificities but consistently resulted in higher estimates of Δ (Figures 2–5, Table 1, Table 2).

DISCUSSION

Differentiated pooling can improve efficiency in settings where subgroups with different test positivity can be reasonably defined. Here, we have illustrated an example where differentiated pooling may be worthwhile in settings where the prevalence of SARS-CoV-2 is predicted by symptoms or known or probable exposure to SARS-CoV-2 versus absence of symptoms and no such probable exposure. Specifically, we see advantages to this approach when testing is required for both symptomatic and unvaccinated individuals, where there are at least 2 groups of easily distinguishable individuals being tested, and group status is predictive of infection. In a university setting, our results suggest between 0.03 and 3.21 more results could be obtained per test kit used when using differentiated versus traditional pooling. Under a broader range of settings, up to 3.97 more results could be obtained per test kit used.

The demand for SARS-CoV-2 testing continues to exceed test supply in numerous locations across the globe (7, 8, 17, 18, 45–47). In most cases, same-day testing for symptomatic or exposed individuals remains unavailable. High demand for testing has placed unprecedented strains on health-care systems broadly, which has contributed to test result processing times often exceeding 4–5 days (8, 45, 47). Given

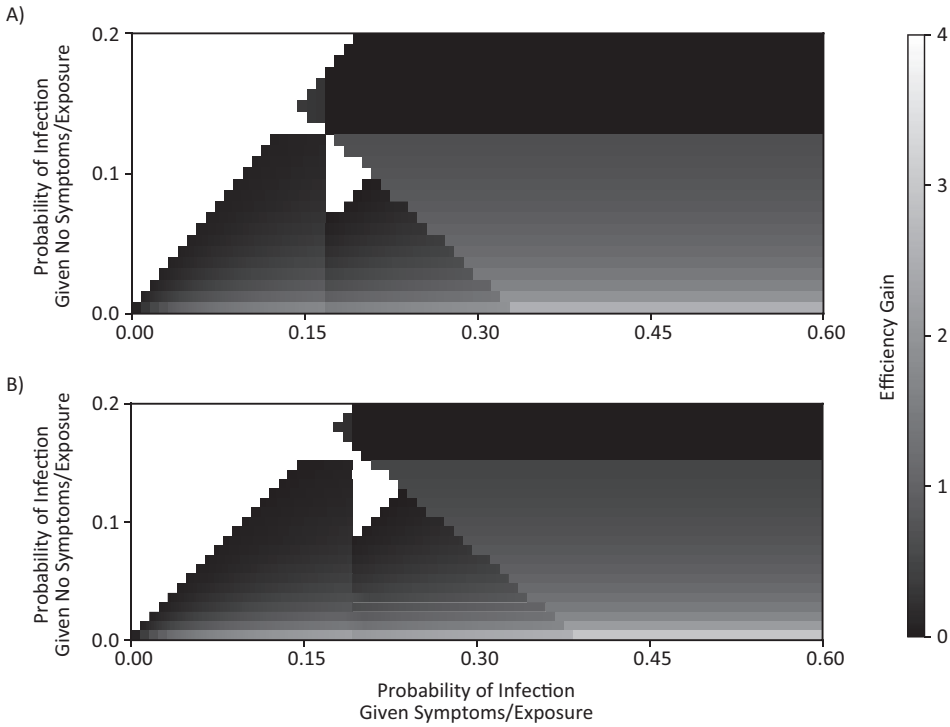

Figure 4. Improvement in efficiency, or additional results obtained per test kit used (Δ) through implementation of a differentiated versus traditional pooling strategy in settings with a probability of infection given symptomatic or exposed ($P(D|G = 1)$) ranging from 0% to 60%, a probability of infection given asymptomatic and unexposed ($P(D|G = 0)$) ranging from 0% to 20%, and a probability of being symptomatic or exposed in the population being tested ($P(G = 1)$) of 50%. A) Assay sensitivity and specificity were set to 0.75 and 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals. B) Assay sensitivity and specificity were set to 0.85 and 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals. White areas represent scenarios in which $P(D|G = 0)$ is greater than $P(D|G = 1)$ or traditional pooling is more efficient than differentiated pooling (i.e., $\Delta < 0$).

the short duration of viral shedding in individuals with less severe COVID-19 (48, 49), results may be far less relevant to public health efforts a week or more after symptom onset (29). These considerations suggest that individual-specimen testing strategies are ineffective at meeting current testing needs, and simple, easy-to-implement strategies for improving testing efficiency are needed.

Many proposed SARS-CoV-2 testing strategies, including other pooled testing strategies proposed in the literature, are suboptimal for various reasons. Traditional pooling and other multistage hierarchical testing strategies originally proposed by Dorfman (27) improve testing efficiency when compared with individual-specimen testing strategies. However, these gains are substantially diminished in higher-prevalence settings (34). Other pooling strategies, such as the P-BEST strategy (50), slice testing or hypercube strategy (51), and novel context-sensitive approaches (52), can improve testing efficiency beyond what is anticipated through traditional pooling but are logistically difficult to implement in real-world settings. The differentiated pooling strategy considered here can be easily employed in settings where there are separate screening locations for symptomatic or exposed individuals and asymptomatic and unexposed individuals (e.g., universities), or in settings utilizing symptom or exposure-based risk-screening tools. Using this approach, unique testing barcodes that indicate the appropriate specimen testing group could be generated at the time of specimen collection, reducing the operational complexity of the strategy.

Other variables, such as vaccination status and type of testing location (e.g., diagnostic center versus screening center for asymptomatic individuals), may also be used to define subgroups in a differentiated pooled testing program, provided the grouping variables are reliable predictors of test positivity. Pool sizes could be further optimized for more than 2 groups (e.g., those who self-report as exposed or symptomatic and unvaccinated; those who self-report as exposed or symptomatic and vaccinated; those who self-report as asymptomatic, unexposed, and vaccinated; and those who self-report as asymptomatic, unexposed, and unvaccinated) provided the test-positive probability of each group is distinct and the characteristic used to differentiate each group is a reliable predictor of infection. This approach could further improve the efficiency of a differentiated pooling strategy.

In addition to these advantages, differentiated pooling can reduce result turnaround time, similar to other pooling strategies (19, 31, 53, 54). Given that a finite number

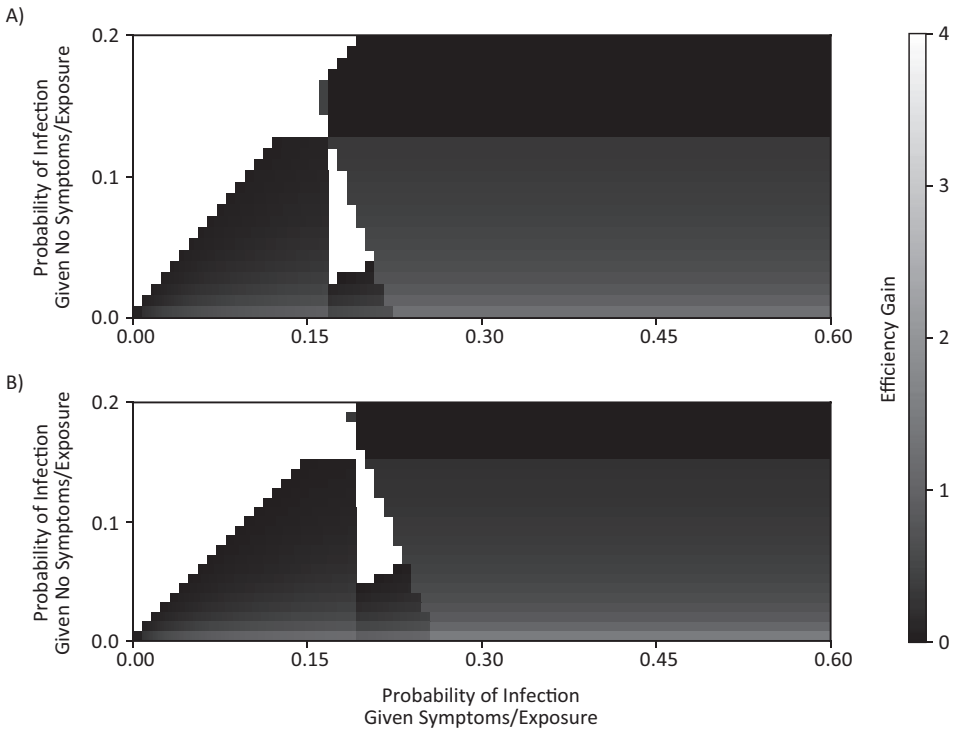

Figure 5. Improvement in efficiency, or additional results obtained per test kit used (Δ) through implementation of a differentiated versus traditional pooling strategy in settings with a probability of infection given symptomatic or exposed ($P(D|G = 1)$) ranging from 0% to 60%, a probability of infection given asymptomatic and unexposed ($P(D|G = 0)$) ranging from 0% to 20%, and a probability of being symptomatic or exposed in the population being tested ($P(G)$) of 75%. A) Assay sensitivity and specificity were set to 0.75 and 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals. B) Assay sensitivity and specificity were set to 0.85 and 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals. White areas represent scenarios in which $P(D|G = 0)$ is greater than $P(D|G = 1)$ or traditional pooling is more efficient than differentiated pooling (i.e., $\Delta < 0$).

of assays can be performed in a period of time, pooling combines samples that would otherwise be processed individually, thereby collapsing samples in the testing queue. Depending on pool size and the number of pools that screen positive, even accounting for subsequent individual testing of samples in pools that screen positive, the total number of assays processed can be smaller than in the absence of pooled testing (especially in the event of larger pool sizes and a smaller proportion of pools screening positive). This can eliminate bottlenecks in result processing that have been frequently observed in settings using an individual-specimen testing strategy (8, 45, 47), thereby improving population-level result turnaround time. Moreover, this approach allows for individual-specimen testing in groups where the prevalence of infection is relatively high while still allowing for pooling in groups where the prevalence of infection is relatively low and pools are less likely to screen positive.

As noted, SARS-CoV-2 viral titer among symptomatic and infected individuals may be higher than that among asymptomatic and infected individuals. Therefore, test sensitivity may differ and the dilution effects of pooling may be more extreme among the asymptomatic and unexposed group. The approach and efficiency calculations described here allow for differential sensitivity and specificity by groups. Ultimately, the relatively low assumed performance of the utilized assay among asymptomatic and unexposed individuals resulted in few scenarios where Δ was negative (i.e., traditional pooling was more efficient compared with differentiated pooling). While the differentiated pooling strategy was not beneficial in these instances, we readily identified such scenarios, further highlighting the applicability of our approach to the establishment of evidence-informed, tailored testing strategies.

In summation, differentiated pooling improves efficiency in testing settings where strong predictors of infection define easily distinguishable groups of people. Here we have demonstrated the improvements obtained through implementation of a differentiated versus traditional pooling strategy in settings such as universities and workplaces and where the prevalence of SARS-CoV-2 in symptomatic or exposed individuals is high, the prevalence of SARS-CoV-2 in asymptomatic and unexposed individuals is low, and the proportion of individuals in the testing population who self-report as symptomatic or exposed is at least 10%. Drawing on existing testing program setup and symptom and exposure screening tools, differentiated pooling imposes a minimal increase in operational complexity, offering

Table 1. Optimal Pool Sizes Among Symptomatic or Exposed Individuals, Asymptomatic and Unexposed Individuals, and the Testing Population Overall, With Corresponding Estimates of Additional Results Obtained per Test Kit Used Through Implementation of a Differentiated Versus Traditional Pooling Strategy, Assuming Conservative Assay Performance Parameters[a]

$P(G=1)$[b]	$P(D\|G=1)$[b]	Optimal Pool Size Among Symptomatic or Exposed	$\varphi_{G=1}$[b]	$P(D\|G=0)$[b]	Optimal Pool Size Among Asymptomatic and Unexposed	$\varphi_{G=0}$[b]	ψ[b]	$P(D)$[b]	Optimal Pool Size in Overall Testing Population	φ[b]	Δ[b]
0.10	0.05	7	2.65	0.01	19	6.06	5.72	0.01	15	4.99	0.74
0.10	0.10	6	1.98	0.03	12	4.04	3.83	0.04	10	3.48	0.35
0.10	0.20	1	1	0.05	10	3.31	3.08	0.06	8	2.75	0.33
0.10	0.30	1	1	0.07	9	2.90	2.71	0.09	7	2.38	0.34
0.10	0.40	1	1	0.09	8	2.64	2.47	0.12	7	2.14	0.34
0.10	0.50	1	1	0.11	8	2.45	2.30	0.11	1	1	1.30
0.10	0.60	1	1	0.13	25	2.33	2.22	0.18	1	1	1.20
0.50	0.05	7	2.65	0.01	19	6.06	4.36	0.03	9	3.33	0.98
0.50	0.10	6	1.98	0.03	12	4.04	3.01	0.06	7	2.48	0.52
0.50	0.20	1	1	0.05	10	3.31	2.16	0.12	6	1.88	0.28
0.50	0.30	1	1	0.07	9	2.90	1.95	0.18	1	1	0.95
0.50	0.40	1	1	0.09	8	2.64	1.82	0.24	1	1	0.82
0.50	0.50	1	1	0.11	8	2.45	1.72	0.30	1	1	0.72
0.50	0.60	1	1	0.13	25	2.33	1.66	0.36	1	1	0.66
0.75	0.05	7	2.65	0.01	19	6.06	3.51	0.04	8	2.95	0.56
0.75	0.10	6	1.98	0.03	12	4.04	2.49	0.08	6	2.19	0.31
0.75	0.20	1	1	0.05	10	3.31	1.58	0.16	5	1.65	−0.07
0.75	0.30	1	1	0.07	9	2.90	1.48	0.24	1	1	0.48
0.75	0.40	1	1	0.09	8	2.64	1.41	0.32	1	1	0.41
0.75	0.50	1	1	0.11	8	2.45	1.36	0.40	1	1	0.36
0.75	0.60	1	1	0.13	25	2.33	1.33	0.48	1	1	0.33

[a] Assay performance parameters were as follows: sensitivity of 0.75 and specificity of 0.95 among symptomatic or exposed individuals and 0.50 and 0.95 among asymptomatic and unexposed individuals.

[b] $P(G=1)$ indicates the probability of being symptomatic or exposed; $P(D|G=1)$ indicates the prevalence of infection among symptomatic or exposed; $\varphi_{G=1}$ indicates the results obtained per test kit used in pooled testing strategy among symptomatic or exposed; $P(D|G=0)$ indicates the prevalence of infection among asymptomatic and unexposed; $\varphi_{G=0}$ indicates the results obtained per test kit used in pooled testing strategy among asymptomatic and unexposed; ψ indicates the results obtained per test kit used in differentiated pooling strategy; $P(D)$ indicates prevalence of infection in testing population overall; φ indicates the results obtained per test kit used in traditional pooling strategy in testing population overall; Δ indicates the change in efficiency, or additional results obtained per test kit used, through implementation of differentiated pooling strategy versus traditional pooling strategy in testing population overall.

Table 2. Optimal Pool Sizes Among Symptomatic or Exposed Individuals, Asymptomatic and Unexposed Individuals, and the Testing Population Overall, With Corresponding Estimates of Additional Results Obtained per Test Kit Used Through Implementation of a Differentiated Versus Traditional Pooling Strategy, Assuming Less Conservative Assay Performance Parameters[a]

$P(G=1)$[b]	$P(D\|G=1)$[b]	Optimal Pool Size Among Symptomatic or Exposed	$\varphi_{G=1}$[b]	$P(D\|G=0)$[b]	Optimal Pool Size Among Asymptomatic and Unexposed	$\varphi_{G=0}$[b]	ψ[b]	$P(D)$[b]	Optimal Pool Size in Overall Testing Population	φ[b]	Δ[b]
0.10	0.05	6	2.67	0.01	16	6.91	6.48	0.01	13	5.49	1.00
0.10	0.10	5	1.93	0.03	10	4.13	3.91	0.04	8	3.52	0.38
0.10	0.20	4	1.40	0.05	8	3.25	3.07	0.06	7	2.68	0.38
0.10	0.30	1	1	0.07	7	2.79	2.61	0.09	6	2.27	0.34
0.10	0.40	1	1	0.09	6	2.48	2.33	0.12	5	2.01	0.32
0.10	0.50	1	1	0.11	6	2.27	2.15	0.15	5	1.83	0.31
0.10	0.60	1	1	0.13	6	2.11	2.0	0.18	1	1	1.00
0.50	0.05	6	2.67	0.01	16	6.91	4.79	0.03	8	3.51	1.28
0.50	0.10	5	1.93	0.03	10	4.13	3.03	0.06	6	2.46	0.57
0.50	0.20	4	1.40	0.05	8	3.25	2.33	0.12	5	1.8	0.53
0.50	0.30	1	1	0.07	7	2.79	1.89	0.18	4	1.51	0.39
0.50	0.40	1	1	0.09	6	2.48	1.74	0.24	1	1	0.74
0.50	0.50	1	1	0.11	6	2.27	1.64	0.30	1	1	0.64
0.50	0.60	1	1	0.13	6	2.11	1.56	0.36	1	1	0.56
0.75	0.05	6	2.67	0.01	16	6.91	3.73	0.04	7	3.01	0.73
0.75	0.10	5	1.93	0.03	10	4.13	2.48	0.08	5	2.14	0.33
0.75	0.20	4	1.4	0.05	8	3.25	1.87	0.16	4	1.56	0.30
0.75	0.30	1	1	0.07	7	2.79	1.45	0.24	1	1	0.45
0.75	0.40	1	1	0.09	6	2.48	1.37	0.32	1	1	0.37
0.75	0.50	1	1	0.11	6	2.27	1.32	0.40	1	1	0.32
0.75	0.60	1	1	0.13	6	2.11	1.28	0.48	1	1	0.28

[a] Assay performance parameters were as follows: sensitivity of 0.85 and specificity of 0.99 among symptomatic or exposed individuals and 0.60 and 0.99 among asymptomatic and unexposed individuals.

[b] $P(G=1)$ indicates the probability of being symptomatic or exposed; $P(D|G=1)$ indicates the prevalence of infection among symptomatic or exposed; $\varphi_{G=1}$ indicates the results obtained per test kit used in pooled testing strategy among symptomatic or exposed; $P(D|G=0)$ indicates the prevalence of infection among asymptomatic and unexposed; $\varphi_{G=0}$ indicates the results obtained per test kit used in pooled testing strategy among asymptomatic and unexposed; ψ indicates the results obtained per test kit used in differentiated pooling strategy; $P(D)$ indicates prevalence of infection in testing population overall; φ indicates the results obtained per test kit used in traditional pooling strategy in testing population overall; Δ indicates the change in efficiency, or additional results obtained per test kit used, through implementation of differentiated pooling strategy versus traditional pooling strategy in testing population overall.

a simple-to-implement opportunity to conserve critical testing resources. Broadly, this approach holds the potential to increase SARS-CoV-2 testing capacity and should be considered a viable option in testing program planning and implementation across the United States and globally.

ACKNOWLEDGMENTS

Author affiliations: Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States (Lindsey M. Filiatreau, Paul N. Zivich, Jessie K. Edwards, Grace E. Mulholland, Ryan Max, Daniel Westreich); Department of Psychiatry, School of Medicine, Washington University in St. Louis, St. Louis, Missouri, United States (Lindsey M. Filiatreau); International Center for Child Health and Development, Brown School, Washington University in St. Louis, St. Louis, Missouri, United States (Lindsey M. Filiatreau); Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States (Paul N. Zivich); and Gillings Center for Coronavirus Testing, Screening, and Surveillance, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States (Jessie K. Edwards, Daniel Westreich).

This work was supported by ViiV Healthcare (L.M.F.), the National Institutes of Health (grants T37MD014218—L.M.F.; T32-HD091058—P.N.Z.; T32-AI007001—P.N.Z.; K01AI125087—J.K.E.), and the University of North Carolina at Chapel Hill, with funding from the North Carolina Coronavirus Relief Fund established and appropriated by the North Carolina General Assembly (J.K.E., D.W.).

Code utilized in this analysis is accessible at https://github.com/pzivich/publications-code.

This work was presented at the 2021 Society for Epidemiologic Research Annual Meeting (virtual), June 22–25, 2021.

Conflict of interest: none declared.

REFERENCES

1. Ahmad FB, Anderson RN. The leading causes of death in the US for 2020. *JAMA*. 2021;325(18):1829–1830.
2. Kirenga BJ, Byakika-Kibwika P. Excess COVID-19 mortality among critically ill patients in Africa. *Lancet.* 2021; 397(10288):1860–1861.
3. Institute of Health Metrics and Evaluation. COVID 19 projections—global. https://covid19.healthdata.org/global?view=cumulative-deaths&tab=compare. Accessed July 16, 2021.
4. Liang LL, Tseng CH, Ho HJ, et al. Covid-19 mortality is negatively associated with test number and government effectiveness. *Sci Rep.* 2020;10(1):1–7.
5. Black JRM, Bailey C, Przewrocka J, et al. COVID-19: the case for health-care worker screening to prevent hospital transmission. *Lancet.* 2020;395(10234):1418–1420.
6. Studdert DM, Hall MA. Disease control, civil liberties, and mass testing—calibrating restrictions during the Covid-19 pandemic. *N Engl J Med.* 2020;383(2):102–104.
7. Beeching NJ, Fletcher TE, Beadsworth MBJ. Covid-19: testing times. *BMJ*. 2020;369:m1403.
8. Lazer D, Baum MA, Quintana A, et al. The state of the nation: a 50-state COVID-19 survey report #8: failing the test: waiting times for COVID diagnostic tests across the US. 2020 https://www.kateto.net/covid19/COVID19%20CONSORTIUM%20REPORT%208%20TEST%20JULY%202020.pdf. Accessed August 21, 2020.
9. Sunjaya AF, Sunjaya AP. Pooled testing for expanding COVID-19 mass surveillance. *Disaster Med Public Health Prep.* 2020;14(3):e42–e43.
10. Denny TN, Andrews L, Bonsignori M, et al. Implementation of a pooled surveillance testing program for asymptomatic SARS-CoV-2 infections on a college campus—Duke University, Durham, North Carolina, August 2–October 11, 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(46): 1743–1747.
11. Paltiel AD, Zheng A, Walensky RP. Assessment of SARS-CoV-2 screening strategies to permit the safe reopening of college campuses in the United States. *JAMA Netw Open.* 2020;3(7):e2016818.
12. Williams SN, Yamey G. How universities can make reopening safer this autumn. *BMJ*. 2021;374:n2019.
13. UNC Chapel Hill. Carolina Together: testing. https://carolinatogether.unc.edu/carolina-together-testing-program/. Accessed September 23, 2021.
14. Tromberg BJ, Schwetz TA, Pérez-Stable EJ, et al. Rapid scaling up of covid-19 diagnostic testing in the United States—the NIH RADx Initiative. *N Engl J Med.* 2020; 383(11):1071–1077.
15. McMahon DE, Peters GA, Ivers LC, et al. Global resource shortages during COVID-19: bad news for low-income countries. *PLoS Negl Trop Dis.* 2020;14(7):e0008412.
16. Behnam M, Dey A, Gambell T, et al. COVID-19: overcoming supply shortages for diagnostic testing. 2020. https://www.mckinsey.com/industries/pharmaceuticals-and-medical-products/our-insights/covid-19-overcoming-supply-shortages-for-diagnostic-testing#. Accessed August 1, 2021.
17. Stolberg SG, LaFraniere S. With omicron, U.S. testing capacity faces intense pressure. *New York Times.* 2021; https://www.nytimes.com/2021/12/17/us/politics/us-covid-tests-omicron.html. Accessed December 20, 2021.
18. Anthes E. As antiviral pills arrive, can testing keep up?. *New York Times.* 2021; https://www.nytimes.com/2021/11/29/health/covid-pill.html. Accessed December 20, 2021.
19. Pilcher CD, Westreich D, Hudgens MG. Group testing for severe acute respiratory syndrome-coronavirus 2 to enable rapid scale-up of testing and real-time surveillance of incidence. *J Infect Dis.* 2020;222(6):903–909.
20. Ben-Ami R, Klochendler A, Seidel M, et al. Large-scale implementation of pooled RNA extraction and RT-PCR for SARS-CoV-2 detection. *Clin Microbiol Infect.* 2020;26(9): 1248–1253.
21. Abdalhamid B, Bilder CR, McCutchen EL, et al. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. *Am J Clin Pathol.* 2020;153(6):715–718.
22. Eis-Hübinger AM, Hönemann M, Wenzel JJ, et al. Ad hoc laboratory-based surveillance of SARS-CoV-2 by real-time RT-PCR using minipools of RNA prepared from routine respiratory samples. *J Clin Virol.* 2020;127:104381.
23. Hogan CA, Sahoo MK, Pinsky BA. Sample pooling as a strategy to detect community transmission of SARS-CoV-2. *JAMA.* 2020;323(19):1967–1969.

24. Yelin I, Aharony N, Tamar ES, et al. Evaluation of COVID-19 RT-qPCR test in multi sample pools. *Clin Infect Dis.* 2020;71(16):2073–2078.
25. Majid F, Omer SB, Khwaja AI. Optimising SARS-CoV-2 pooled testing for low-resource settings. *Lancet Microbe.* 2020;1(3):e101–e102.
26. Lohse S, Pfuhl T, Berkó-Göttel B, et al. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. *Lancet Infect Dis.* 2020;20(11):1231–1232.
27. Dorfman R. The detection of defective members of large populations. *Ann Math Stat.* 1943;14(4):436–440.
28. Chow WK, Chow CL. A discussion on implementing pooling detection tests of novel coronavirus (SARS-CoV-2) for large population. *Epidemiol Infect.* 2021;149:149.
29. Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Sci Adv*. 2021;7(1):eabd5393.
30. Rewley J. Specimen pooling to conserve additional testing resources when persons' infection status is correlated: a simulation study. *Epidemiology.* 2020;31(6): 832–835.
31. Grobe N, Cherif A, Wang X, et al. Sample pooling: burden or solution? *Clin Microbiol Infect.* 2021;27(9):1212–1220.
32. Chan CW, Kwon S, Matushek SM, et al. Implementation of a sample pooling strategy for the direct detection of SARS-CoV-2 by real-time polymerase chain reaction during the COVID-19 pandemic: an institutional experience. *Am J Clin Pathol.* 2021;156(1):15–23.
33. Westreich DJ, Hudgens MG, Fiscus SA, et al. Optimizing screening for acute human immunodeficiency virus infection with pooled nucleic acid amplification tests. *J Clin Microbiol.* 2008;46(5):1785–1792.
34. Polage CR, Lee MJ, Hubbard C, et al. Assessment of an online tool to simulate the effect of pooled testing for SARS-CoV-2 detection in asymptomatic and symptomatic populations. *JAMA Netw Open.* 2020;3(12): e2031517.
35. McMahan CS, Tebbs JM, Bilder CR. Informative Dorfman screening. *Biometrics.* 2012;68(1):287–296.
36. Lan FY, Lan FY, Filler R, et al. COVID-19 symptoms predictive of healthcare workers' SARS-CoV-2 PCR results. *PLoS One.* 2020;15(6):e0235460.
37. Bateman AC, Mueller S, Guenther K, et al. Assessing the dilution effect of specimen pooling on the sensitivity of SARS-CoV-2 PCR tests. *J Med Virol.* 2021;93(3):1568–1572.
38. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18): 1843–1844.
39. Bisoffi Z, Pomari E, Deiana M, et al. Sensitivity, specificity and predictive values of molecular and serological tests for COVID-19: a longitudinal study in emergency room. *Diagnostics (Basel).* 2020;10(9):669.
40. Giri B, Pandey S, Shrestha R, et al. Review of analytical performance of COVID-19 detection methods. *Anal Bioanal Chem.* 2021;413(1):35–48.
41. Kissler SM, Fauver JR, Mack C, et al. Viral dynamics of acute SARS-CoV-2 infection and applications to diagnostic and public health strategies. *PLoS Biol.* 2021;19(7): e3001333.
42. Zhang Z, Bi Q, Fang S, et al. Insight into the practical performance of RT-PCR testing for SARS-CoV-2 using serological data: a cohort study. *Lancet Microbe.* 2021; 2(2):e79–e87.
43. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis.* 2020;20(6): 656–657.
44. Fu Y, Li Y, Guo E, et al. Dynamics and correlation among viral positivity, seroconversion, and disease severity in COVID-19 : a retrospective study. *Ann Intern Med.* 2020; 174(4):453–461.
45. Torres I, Sippy R, Sacoto F. Assessing critical gaps in COVID-19 testing capacity: the case of delayed results in Ecuador. *BMC Public Health*. 2021;21(1):637.
46. Mercer TR, Salit M. Testing at scale during the COVID-19 pandemic. *Nat Rev Genet.* 2021;22(7):415–426.
47. The COVID-19 testing debacle. *Nat Biotechnol.* 2020;38(6): 653.
48. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Med.* 2020;26(5):672–675.
49. Huang JT, Ran RX, Lv ZH, et al. Chronological changes of viral shedding in adult inpatients with COVID-19 in Wuhan, China. *Clin Infect Dis.* 2020;71(16):2158–2166.
50. Shental N, Levy S, Wuvshet V, et al. Efficient high-throughput SARS-CoV-2 testing to detect asymptomatic carriers. *Sci Adv.* 2020;6(37):5961–5972.
51. Mutesa L, Ndishimye P, Butera Y, et al. A pooled testing strategy for identifying SARS-CoV-2 at low prevalence. *Nature.* 2021;589(7841):276–280.
52. Deckert A, Bärnighausen T, Kyei NNA. Simulation of pooled-sample analysis strategies for covid-19 mass testing. *Bull World Health Organ.* 2020;98(9):590–598.
53. Agoti CN, Mutunga M, Lambisia AW, et al. Pooled testing conserves SARS-CoV-2 laboratory resources and improves test turn-around time: experience on the Kenyan coast. *Wellcome Open Res.* 2021;5:186.
54. Sahajpal NS, Mondal AK, Njau A, et al. Proposal of RT-PCR–based mass population screening for severe acute respiratory syndrome coronavirus 2 (coronavirus disease 2019). *J Mol Diagn.* 2020;22(10):1294–1299.