Title

Optimal uses of pooled testing for COVID-19 incorporating imperfect test performance and pool dilution effect: An application to congregate settings in Los Angeles County

Journal

Journal of Medical Virology, 93(9)

ISSN

0146-6615

Authors

Nianogo, Roch A
Emeruwa, I Obi
Gounder, Prabhu
et al.

Publication Date

2021-09-01

DOI

10.1002/jmv.27054

Peer reviewed

Received: 24 March 2021 | Revised: 22 April 2021 | Accepted: 27 April 2021

DOI: 10.1002/jmv.27054

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Optimal uses of pooled testing for COVID-19 incorporating imperfect test performance and pool dilution effect: An application to congregate settings in Los Angeles County

Roch A. Nianogo MD, PhD[1,2] | I. Obi Emeruwa MD, MBA[3,4,5] | Prabhu Gounder MD, MPH[6] | Vladimir Manuel MD[5] | Nathaniel W. Anderson BA[2,3,4] | Tony Kuo MD, MSHS[1,4,7] | Moira Inkelas PhD, MPH[3,4] | Onyebuchi A. Arah MD, PhD, DSc, MPH, MSc[1,2,8]

[1]Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles (UCLA), Los Angeles, California, USA

[2]California Center for Population Research, Los Angeles, California, USA

[3]Department of Health Policy and Management, UCLA Fielding School of Public Health, Los Angeles, California, USA

[4]UCLA Clinical and Translational Science Institute, Los Angeles, California, USA

[5]Division of Pulmonary and Critical Care Medicine, UCLA David Geffen School of Medicine, Los Angeles, California, USA

[6]Los Angeles County Department of Public Health (LACDPH), Los Angeles, California, USA

[7]Department of Family Medicine, David Geffen School of Medicine, Los Angeles, California, USA

[8]Department of Statistics, College of Letters and Science, UCLA, Los Angeles, California, USA

Correspondence

Roch A. Nianogo, MD, PhD, Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles (UCLA), Los Angeles, CA 90095, USA.
Email: niaroch@ucla.edu

ABSTRACT

Introduction: Pooled testing is a potentially efficient alternative strategy for COVID-19 testing in congregate settings. We evaluated the utility and cost-savings of pooled testing based on imperfect test performance and potential dilution effect due to pooling and created a practical calculator for online use.

Methods: We developed a 2-stage pooled testing model accounting for dilution. The model was applied to hypothetical scenarios of 100 specimens collected during a one-week time-horizon cycle for varying levels of COVID-19 prevalence and test sensitivity and specificity, and to 338 skilled nursing facilities (SNFs) in Los Angeles County (Los Angeles) (data collected and analyzed in 2020).

Results: Optimal pool sizes ranged from 1 to 12 in instances where there is a least one case in the batch of specimens. 40% of Los Angeles SNFs had more than one case triggering a response-testing strategy. The median number (minimum; maximum) of tests performed per facility were 56 (14; 356) for a pool size of 4, 64 (13; 429) for a pool size of 10, and 52 (11; 352) for an optimal pool size strategy among response-testing facilities. The median costs of tests in response-testing facilities were $8250 ($1100; $46,100), $6000 ($1340; $37,700), $6820 ($1260; $43,540), and $5960 ($1100; $37,380) when adopting individual testing, a pooled testing strategy using pool sizes of 4, 10, and optimal pool size, respectively.

Conclusions: Pooled testing is an efficient strategy for congregate settings with a low prevalence of COVID-19. Dilution as a result of pooling can lead to erroneous false-negative results.

KEYWORDS

congregate setting, cost saving, COVID-19, dilution, pooled testing, skilled nursing facilities

1 | INTRODUCTION

Testing is essential for monitoring and mitigating the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19).[1,2] Due to nationwide shortages in testing reagents and supplies, testing capacity in the United States and worldwide has been constrained. These constraints lengthened the turnaround time for receiving the diagnostic result as demand for testing increased. Public health guidance for congregate settings such as skilled nursing facilities (SNFs) required a volume of testing that is expensive and that could worsen the turnaround time for SARS-CoV-2 test results for residents of these facilities and the general public.[3,4] Delayed results can slow the identification of outbreaks in these facilities and limit the effect of other mitigation efforts.[5–7] Therefore, cost-effective methods to reduce turnaround time for obtaining test results and for maintaining appropriate testing frequency are urgently needed.

Pooled testing is one such method that has been used to optimize limited testing resources and has been approved by the US Food and Drug Administration (FDA).[8–11] Pooled testing, or group testing, was first proposed in 1943 by Robert Dorfman as a solution to the aforementioned problem that would require fewer chemical analyses than would individual testing for testing a large population.[9] Briefly, pooled testing involves grouping individual test specimens into pools which are then processed. If a pool is negative, then there is no further testing of the individual specimens in that pool. If a pool is positive, then all individual specimens in that pool are processed.[9] Research is ongoing to perfect this method.[12–14] The demand for testing in the COVID-19 pandemic has rekindled the need to revisit this strategy. In their recent study, Cherif et al.[15] described an epidemiologic model that simulated the impact of pooled testing based on regional disease prevalence and SARS-CoV-2 real-time reverse transcription-polymerase chain reaction (PCR) testing characteristics. The authors concluded that a pooled testing strategy is an improvement over individual testing in settings where there is a COVID-19 prevalence of less than 30 percent and a pool size that varies with the inverse of the square root of the prevalence and test sensitivity. Although this study shed some light on pooled testing for COVID-19, it did not thoroughly address the potential impact of the dilution effect due to pooling, that is, the decrease in test sensitivity as a result of pooled testing.[16]

We designed the current study to address this additional question by evaluating the impact of imperfect testing performance and pooling dilution on the number of tests needed as well as on the potential resulting cost savings, first in a hypothetical scenario and second using actual prevalence data from Los Angeles SNFs. In addition, we developed an analytic 'calculator' with an accompanying online tool to estimate pool sizes that may be needed at these settings, comparing the potential tradeoffs in terms of test swabs, reagents, supplies and performance, and cost savings. This study can advance our understanding of how pooled testing could be used to monitor and manage SARS-CoV-2 infection in congregate settings, especially for SNFs in Los Angeles and throughout the U.S.[4,17] The study focuses on SNFs because these patients are among the most vulnerable at-risk populations in the U.S. and worldwide - accounting for approximately one-third to one-half of COVID-19 deaths.[17] The Centers for Disease Control and Prevention (CDC) recently issued guidance that encourages use of pooled testing in such facilities.[3,4,18]

2 | METHODS

2.1 | Pooled testing overview

We developed a hierarchical 2-stage pooled Dorfman testing calculator with an accompanying online tool for estimating the optimal pool size that minimizes the number of tests needed. This tool allows for several input parameters highlighted in the box below (number of specimens, a priori COVID-19 prevalence in the specimens, sensitivity, specificity, and pool size). Let T be a random variable representing the number of tests needed.

Box: Definition of parameters

Symbol	Description
p	a priori COVID-19 prevalence in the specimens
n	Total number of specimens collected
s	Size of the pool
k = n/s	Number of pools
se	Sensitivity of the test
se_d	Diluted sensitivity of the test
sp	Specificity of the test
$p_{pos\_specimen} = p^*se + (1 - sp)^*(1 - p)$	Probability that a specimen tests positive
$p_{pos\_pools} = 1 - (1 - p_{pos\_specimen})^s$	Probability that a pool tests positive
$k^{+\cdot} = k \bullet p_{pos\_pools}$	Expected number of positive pools
	Expected total number needed to test (NNT)

The expected total number needed to test (NNT):

$$E(T) = k + k^{+} \bullet s = k + p_{pos\_pools} \bullet k \bullet s = n/s + n^* p_{pos\_pools} = n \bullet (1/s + p_{pos\_pools})$$

or more specifically:

$$E(T|n, p, se, sp, s) = ns + n \cdot [1 - (1 - (p^*se + (1 - sp)^*(1 - p)))^s] \quad (1)$$

That is, E(T|n, p, se, sp,s) is equal to the number of tests in the first stage: k = n/s added to the number of tests in the second stage: $k^{+} \bullet s = p_{pos\_pools} \bullet k \bullet s = p_{pos\_pools} \bullet n$.

The optimal pool size would be the size of the pool, s_0, which minimizes the total number of tests needed. For comparison, the number of tests needed in an individual testing strategy where all individual specimens are initially tested ($E(T) = k \bullet s = n$) is provided.

2.2 | Development of the dilution model

We searched the literature for the existing dilution model for pooled testing for COVID-19 to inform our pooled testing strategy. In the absence of current dilution models for COVID-19, we developed a preliminary prediction model of decreased sensitivity as a function of pool size. Several studies have suggested that test sensitivity decreases with increasing pool sizes, but few have developed an easy-to-implement prediction dilution model as a function of pool sizes for SARS-CoV-2. We used limited data from Bateman et al who estimated that pools of 5, 10, and 50 specimens led to false-negative rates of 7%, 9%, and 19%, respectively.[16] Based on the shape of these data, we hypothesized that the model would follow a decaying nonlinear trend as a function of pool size. We then tested several models: linear, logarithmic, exponential, and polynomial models. The log transformation model performed the best in terms of the following metrics: R-squared, root-mean-squared error (RMSE), Akaike information criterion (AIC), and Bayesian Information Criterion (BIC) (Appendix Table 1 and Appendix Figure 1). The final model had the form $E(se_d|\cdot) = \alpha + \beta \cdot \log(s)$ where $E(se_d|se, s)$ is the sensitivity function as a function of the pool size s and α and β are two calibration parameters. The calibration of the model, which amounted to an optimization problem that can be solved in the R software, resulted in α^* = starting sensitivity (se), and $\beta^* = -4.30$.

$$E(se_d \mid se, s) = se - 4.30 \cdot \log(s). \quad (2)$$

Where se_d is the diluted sensitivity due to pooling.

We used this prediction model to estimate the decreased sensitivity as a result of pool size. For instance, when the pool size = 1 (i.e., reverts to individual testing), $E(se_d \mid se, S=1) = se$, that is, the sensitivity is unchanged.

2.3 | False-negative rate due to dilution

Public health professionals would be concerned about the potential for missing a pool and therefore failing to test the individual specimens within that pool. A resulting decreasing sensitivity leads to an increasing false-negative rate and thus a corresponding erroneous lower number of tests estimated. From Equation 1 and Equation 2, we can ascertain the number of cases that would be missed when using a pooling strategy if dilution were to occur. The formula for estimating the number of missed specimens would be as follows. For a given pool size, the number of cases that would be missed in a pooled testing strategy is given by Equation 3:

$$\begin{aligned}\text{Number of cases missed} = (1\text{-se}) \\ *[E(T \mid n, p, se, sp, s) \\ - E(T \mid n, p, \widehat{se}_d, sp, s)].\end{aligned} \quad (3)$$

Where $[E(T|n,p, se, sp, s) - E(T|n, p, \widehat{se}_d, sp, s)]$ is the difference in the number needed to test for given parameters: n,p, se, sp,s.

2.4 | Application

We first applied these formulas and algorithms to a hypothetical scenario and then to Los Angeles County SNFs.

2.5 | Hypothetical scenario

For illustration, generalizability, and for quick decision-making, we provided a chart of test volume and cost-saving according to varying degrees of sensitivity, specificity, prevalence, and pool sizes (Table 1). This chart was built using 100 specimens.

2.6 | Los Angeles County SNFs

The data for this application came from the CDC's National Healthcare Safety Network (NHSN) accessed through the Centers for Medicare and Medicaid Services dashboard on July 7th, 2020.[19] Our study utilized cross-sectional data from 338 Los Angeles SNFs which included counts of COVID-19 cases as well as resident volume and capacity. The Los Angeles County Department of Public Health adopted a strategy of testing 10% of residents and 25% of staff members if there were no COVID-19 cases in the SNF during the previous 14 days (i.e., surveillance testing) or 100% of noncase residents and staff members if there were one or more cases in the previous 14 days (i.e., response testing).[5] To apply our calculator to Los Angeles SNFs, we assumed sensitivity of 85% and a specificity of 95% for the PCR test and a cost of $20 for the test collection kit and $80 for the PCR test (LACDPH, Personal Communication, 2020). The prevalence of COVID-19 in the SNF was calculated as the number of currently isolated residents with suspected or confirmed COVID-19 plus divided by the number of residents in the SNF for that week. The total number of specimens collected included specimens from residents and staff for response or surveillance testing in one week for that specific facility (Figure 1).

All the analyses were conducted in R and the calculator built in R Shinyapp version 3.6.3 and can be found at: https://nianogo.shinyapps.io/pooled_testing.

3 | RESULTS

3.1 | Hypothetical scenario

From Table 1 and Figure 2, we can infer the following: for a given test performance, as prevalence increases, the optimal pool size

TABLE 1 Optimal pool sizes and the corresponding number of tests needed, costs, and cost-savings (n = 100 specimens) incorporating the dilution effect

Sensitivity	Specificity	Prevalence	Optimal pool size	Number of PCR tests in the pooled testing strategy	Number of PCR tests saved relative to individual testing strategy (=100)	Cost of pooled testing strategy[a]	Cost savings relative to individual testing strategy (=$10,000)[a]	Cost favors pool or individual testing[a]	Difference in pool sizes (with dilution vs. without dilution)	Number of false-negative results due to dilution[b]
1	1	0	100	1	99	$2080	$7920	Pool	0	0
1	1	0.01	9	19	81	$3520	$6480	Pool	0	0
1	1	0.05	5	42	58	$5360	$4640	Pool	0	0
1	1	0.1	4	58	42	$6640	$3360	Pool	0	0
1	1	0.15	3	71	29	$7680	$2320	Pool	0	0
1	1	0.2	3	81	19	$8480	$1520	Pool	0	0
1	1	0.25	3	90	10	$9200	$800	Pool	0	0
1	1	0.3	3	97	3	$9760	$240	Pool	−2	NE
1	0.95	0	5	43	57	$5440	$4560	Pool	0	0
1	0.95	0.01	4	47	53	$5760	$4240	Pool	0	0
1	0.95	0.05	4	58	42	$6640	$3360	Pool	0	0
1	0.95	0.1	3	70	30	$7600	$2400	Pool	0	0
1	0.95	0.15	3	80	20	$8400	$1600	Pool	0	0
1	0.95	0.2	3	88	12	$9040	$960	Pool	0	0
1	0.95	0.25	3	96	4	$9680	$320	Pool	0	0
1	0.95	0.3	1	100	0	$10,000	$0	Either	0	0
0.85	1	0	100	1	99	$2080	$7920	Pool	0	0
0.85	1	0.01	12	17	83	$3360	$6640	Pool	−3	NE
0.85	1	0.05	6	38	62	$5040	$4960	Pool	−1	NE
0.85	1	0.1	4	54	46	$6320	$3680	Pool	0	0.15
0.85	1	0.15	4	65	35	$7200	$2800	Pool	−1	NE
0.85	1	0.2	3	75	25	$8000	$2000	Pool	0	0.3
0.85	1	0.25	3	83	17	$8640	$1360	Pool	0	0.3
0.85	1	0.3	3	90	10	$9200	$800	Pool	0	0.3
0.85	0.95	0	5	43	57	$5440	$4560	Pool	0	0
0.85	0.95	0.01	5	46	54	$5680	$4320	Pool	0	0
0.85	0.95	0.05	4	56	44	$6480	$3520	Pool	0	0.15
0.85	0.95	0.1	3	67	33	$7360	$2640	Pool	0	0.15
0.85	0.95	0.15	3	75	25	$8000	$2000	Pool	0	0.3
0.85	0.95	0.2	3	83	17	$8640	$1360	Pool	0	0.3
0.85	0.95	0.25	3	90	10	$9200	$800	Pool	0	0.3
0.85	0.95	0.3	3	96	4	$9680	$320	Pool	0	0.3

Note: The number of tests, costs, and cost-savings in these hypothetical scenarios are calculated for a 1-week cycle.

[a]Each test is assumed to cost $20 for the collection kit and $80 for the real-time reverse transcription-polymerase chain reaction (PCR) test.

[b]The number of false-negative results is estimated by calculating the number of false negatives of the difference in the number of tests needed when incorporating dilution vs. not incorporating dilution. Number of cases missed = (1-se)*[E(T|n,p,se,sp,s) − E(T|n,p, $\hat{se}_d$, sp,s)], where, p is the a priori COVID-19 prevalence in the specimens; n, the total number of specimens collected; s, the size of the pool; k, the number of pools; se, the sensitivity of the test; se_d, the diluted sensitivity of the test and the sp, specificity of the test. NE = not estimated (this is so because the pool sizes were different in the dilution and without-dilution scenario).

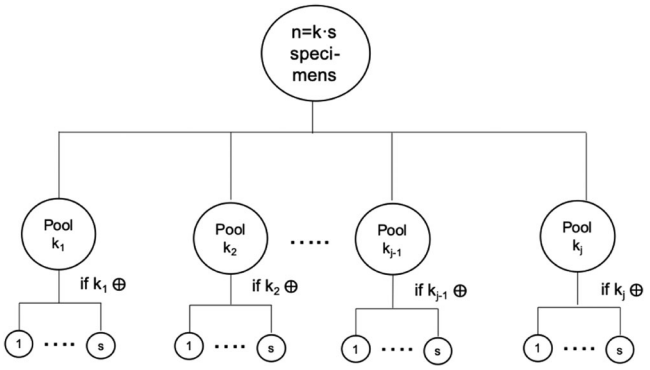

FIGURE 1 Illustration of the Dorfman two-stage hierarchical pooled testing algorithm. Figure adapted from Wang et al.[12] *n* is the number of specimens to be tested, k=n/s is the number of pools and s=n/k is the size of the pool. In stage 1, specimens are divided into k pools. In stage 2, only if pool k_j tests positives, will subsequent tests be done for each specimen of that pool

decreases, and the number of required tests increases. This translates to cost savings of up to $7920 for a batch of 100 specimens, with prevalence ranging from 0% to 30%, test sensitivity of 85% and 100%, and test specificity of 95% and 100%. Optimal pool sizes ranged from 1 to 12 in instances where there is a least one case in the batch of specimens (i.e., prevalence >0). When the prevalence is 0 and test specificity =1, the optimal pool size for a population of 100 specimens would be as expected, 100. Lower prevalence predicted a lower number of tests needed. In this hypothetical scenario, the number of cases missed due solely to dilution appears to be low (less than 1) for a batch of 100 specimens. Uniform pool sizes of 4 tended to yield better return in the number of tests needed as well as cost savings compared to uniform pool sizes of 10. (Appendix Table 3 and Table 4).

3.2 | Los Angeles SNFs scenario

About 40% of SNFs had more than one case triggering a response-testing strategy as described above. In our simulation, the median number (minimum; maximum) of tests performed per facility was 56 (14; 356) for a pool size of 4, 64 (13; 429) for a pool size of 10, and 52 (11; 352) for an optimal pool size strategy among facilities that use response testing. The median costs of tests in response-testing facilities when adopting an individual testing strategy, a pooled testing strategy using pool sizes of 4, 10, and optimal pool size, respectively, would be $8250 ($1100; $46100), $6000 ($1340; $37,700), $6820 ($1260; $43,540) and $5960 ($1100; $37,380) (Table 2). Accounting for the dilution effect of pooling, the expected total number of cases missed due solely to dilution in SNFs response testing was 23 in pooled testing with a pool size of 4, 34 with a pool size of 10, and 17 using an optimal pool size (Table 3).

4 | DISCUSSION

This study evaluated the relative utility in terms of the number of tests saved and the cost (savings) of pooled testing accounting for imperfect test performance and the potential dilution effect due to pooling. In our hypothetical scenarios with varying degrees of prevalence, test performance, and pool size, the optimal pool sizes ranged from 1 to 12 in instances where there was a least one case in the batch of specimens to be tested. In addition, as expected, a lower prevalence of COVID-19 predicted a lower number of tests needed. Conversely, for given test performance, as prevalence increases, the optimal pool size decreases, and the number of tests needed would increase.

Furthermore, the number of cases missed due solely to dilution appears to be low (less than 1) for a batch of 100 specimens. When sensitivity is high (close to 100%), the number of specimens in a batch is small (<100), and when optimal pool sizes are less than 10, there are virtually no cases missed as a result of the dilution effect due to pooling. Uniform pool sizes of 4 tended to yield a better return in the number of tests needed as well as cost savings when compared to uniform pool sizes of 10. In Los Angeles SNFs, using an optimal pool size strategy would require fewer tests needed and therefore lower cost. For these facilities as a group, a uniform pool size of 4 (following the FDA recommendation)[7] yielded an approximately similar number of tests needed and corresponding costs to those when using an optimal pool size strategy, regardless of test performance and prevalence. This is potentially due to the relatively low prevalence of COVID-19 in most Los Angeles SNFs during the study period. Furthermore, if SNFs used a pooled testing strategy during response-testing, the expected total number of cases missed due solely to dilution was lowest when the SNF used an optimal pool size.

Our findings add to those of Cherif et al.[15] by documenting the negative impact of the false-negative rate on resource efficiency gains from pooled testing. Furthermore, the results of the current study are consistent with previous studies indicating that there is little to no dilution effect for pool sizes less than 5.[16,20] Of high importance is the seemingly lower number of tests needed and the corresponding costs when considering the dilution effect (relative to when not considering the dilution effect) that could lead to erroneous conclusions. Fortunately, as previously mentioned, when sensitivity is high, the number of specimens in a batch is small and the pool size is also small (~5), there are virtually no cases missed as a result of the dilution effect due to pooling. It is therefore important to limit pool sizes to a maximum of 5 to maximize the gain in efficiency while minimizing the loss in sensitivity. Therefore, recommendations such as those made by the FDA,[7] encouraging the use of pool sizes of up to 4 are warranted, as was seen in our Los Angeles County SNFs application. Furthermore, even though the number missed per batch for one location could appear relatively small, this number, as seen in this study, can rapidly increase when considering several SNFs altogether—potentially countering efforts to mitigate the transmission of SARS-Cov2 within and across SNFs.

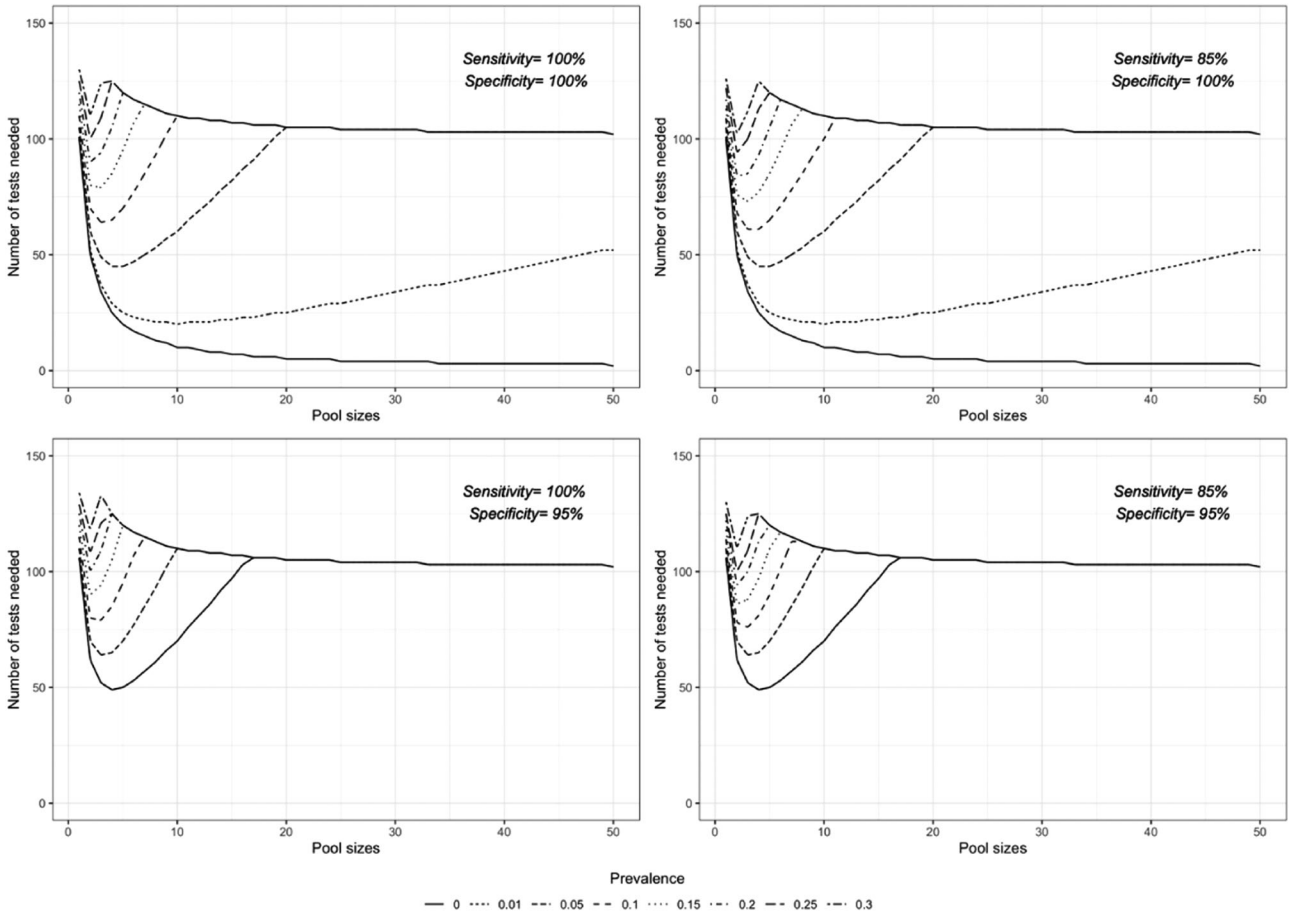

FIGURE 2 Average number of tests needed in the pooled testing strategy as a function of pool size and for the different prevalence of COVID-19 and different test performance ($n = 100$) incorporating the dilution effect of pooling. The figure plots the average number of tests needed as a function of pool sizes for varying levels of prevalence ranging from 0% to 30%, test sensitivity of 85% and 100%, and test specificity of 95% and 100%. The optimal pool size is the size that minimizes the total number of tests needed

Additionally, our analyses reiterated the importance of choosing optimal pool sizes as the best option when considering a pooled testing strategy as it leads to the lowest number of cases missed.

These results altogether emphasize two main points: (1) pooled testing is a viable option when prevalence is low and (2) using an optimal pool size strategy coupled with a test with high sensitivity would ensure the lowest number of false-negative tests. Pooled testing in populations where prevalence is low would maximize efficiency. Public health agencies and SNFs should consider quarantine and other isolation procedures carefully when deciding whether or not to use pooled testing as the initial stage of disease detection. The online calculator provided in this article offers a way to determine an efficient approach to COVID-19 testing under different conditions.

Notably, the epidemiologic context for pooled testing has changed over the course of the COVID-19 pandemic. Pooled testing strategies early in the pandemic were considered as a potential solution for conducting surveillance in the setting of constrained testing capacity.[8–11] Los Angeles did not employ this strategy because high estimated COVID-19 prevalence across the region meant that many pools were likely to be positive. The availability of COVID-19 vaccines and the prioritization of vaccination for SNF residents and staff changed the conditions in Los Angeles[21] with 74% fully vaccinated by mid-April 2021. The COVID-19 vaccine should substantially reduce transmission in SNFs. However, public health testing requirements remain because of uncertainties in the duration of protection and the extent to which vaccines prevent infection and transmission. Such a low-incidence setting in which comprehensive surveillance testing is desired is the ideal scenario in which to consider pooled testing. Additionally, pooled testing is a promising strategy in school systems that are pursuing surveillance testing - prevalence is expected to be low, and the required logistics and burden of individual testing on children could be very high.

Our study is not without limitations. First, we used limited data to calibrate our sensitivity model. Although the final log transformation model outperformed the other tested models in common performance metrics, overfitting could not be excluded. Future studies with larger datasets are needed to confirm these results. Second, data from the Los Angeles SNFs represent only a snapshot of the COVID-19 pandemic at one-time point in a rapidly evolving

TABLE 2 Number of tests performed and associated costs in Los Angeles County skilled nursing facilities incorporating the dilution effect due to pooling (n = 338[a])

Characteristic	Response-test facilities[b], N = 132 (39%)	Surveillance facilities[b], N = 206 (61%)
SNF sizes and cases		
Total number of staff members[c]	6555	7938
Total number of residents[c]	11,133	14,349
Number of staff members per facility[d]	40 (1, 410)	34 (5, 127)
Number staff with suspected/confirmed infection per facility[d]	0.0 (0.0, 3.0)	NA
Percent staff with suspected/confirmed infection per facility[d]	0.00 (0.00, 0.08)	NA
Number of residents per facility[d]	75 (18, 280)	65 (1, 252)
Current residents with suspected/confirmed infection per facility[d]	6 (0, 105)	–
Percent residents with suspected/confirmed infection per facility[d]	0.09 (0.00, 1.00)	–
Tests		
Total number of tests performed using individual testing[c]	12,359	4264
Total number of tests performed using pooled testing (4 specimen/pool)[c]	8252	1958
Total number of tests performed using pooled testing (10 specimen/pool)[c]	9599	2289
Total number of tests performed using pooled testing (optimal pool size)[c]	8043	1912
Number of tests performed per facility using individual testing[d]	82 (11, 461)	19 (12, 55)
Number of tests performed per facility using pooled testing (4 specimen/pool)[d]	56 (14, 356)	9 (6, 24)
Number of tests performed per facility using pooled testing (10 specimen/pool)[d]	64 (13, 429)	10 (7, 28)
Number of tests performed per facility using pooled testing (optimal pool Size)[d]	52 (11, 352)	9 (6, 24)
Costs		
Total cost of tests performed using individual testing[c]	$1,235,900	$426,400
Total cost of tests performed using pooled testing (4 specimen/pool)[c]	$907,340	$241,920
Total cost of tests using pooled testing (10 specimen/pool)[c]	$1,015,100	$268,400
Total cost of tests using pooled testing (optimal pool size)[c]	$890,620	$238,240
Cost of tests per facility using individual testing[d]	$8250 ($1100, $46,100)	$1900 ($1200, $5500)
Cost of tests per facility using pooled testing (4 specimen/pool)[d]	$6000 ($1340, $37,700)	$1,100 ($720, $3020)
Cost of tests per facility using pooled testing (10 specimen/pool)[d]	$6820 ($1260, $43,540)	$1180 ($800, $3340)
Cost of tests per facility using pooled testing (optimal pool size)[d]	$5960 ($1100, $37,380)	$1100 ($720, $3020)

[a]These data were retrieved for July 7th, 2020 (Data accessed on July 7, 2020) and exclude SNFs with missing data on required elements ("Current Isolated COVID+" or "Suspected Residents in Facility") or who did not report any staff members at the SNF in the last 24 h.[15]

[b]California Department of Public Health Mitigation Plan Recommendations for Testing of Health Care Personnel (HCP) and Residents at Skilled Nursing Facilities (SNF).[4]

[c]Sum.

[d]Median (min, max).

health crisis. Third, the cost calculations consider variable costs of testing and do not include fixed costs of establishing a laboratory capable of performing pooled testing.

In summary, testing requirements in SNFs[4] and other congregate settings may continue to strain testing supplies, reagents, and processing capacity. This study revealed that pooled testing is an efficient way to reduce costs and test utilization when SARS-CoV-2 prevalence is low (as is likely to be the case for SNFs as well as other congregate settings such as K-12 school systems), test performance is high, and the pool size is small.

TABLE 3 Number of false-negative tests expected in Los Angeles County skilled nursing facilities if they used pooling assuming a sensitivity of 85% and incorporating the dilution effect due to pooling ($n = 338$[a])

Characteristic	Response-test facilities[b], N = 132 (39%)	Surveillance facilities[b], N = 206 (61%)
Tests		
Total number of false-negative tests expected using individual testing[c]	0	0
Total number of false-negative tests expected using pooled testing (4 specimen/pool)[c]	23	0
Total number of false-negative tests expected using pooled testing (10 specimen/pool)[c]	34	0
Total number of false-negative tests expected using pooled testing (optimal pool size)[c]	17	0
Number of false-negative tests expected per facility using individual testing[d]	0 (0, 0)	0 (0, 0)
Number of false-negative tests expected per facility using pooled testing (4 specimen/pool)[d]	0 (0, 2)	0 (0, 0)
Number of false-negative tests expected per facility using pooled testing (10 specimen/pool)[d]	0 (0, 2)	0 (0, 0)
Number of false-negative tests expected per facility using pooled testing (optimal pool size)[d]	0 (0, 1)	0 (0, 0)

[a]These data were retrieved for July 7th, 2020 and exclude SNFs with missing data on required elements ("Current Isolated COVID+" or "Suspected Residents in Facility") or who did not report any staff members at the SNF in the last 24 h.[15]

[b]California Department of Public Health Mitigation Plan Recommendations for Testing of Health Care Personnel (HCP) and Residents at Skilled Nursing Facilities (SNF).[4]

[c]Sum.

[d]Median (min, max).

5 | CONCLUSIONS

Prior and present studies of pooled testing suggest that this novel testing strategy is a potentially viable approach for monitoring and informing outbreak control in communal environments such as SNFs when the assumed virus/disease prevalence is low. Other congregate settings such as workplaces,[22] residential universities and schools may benefit from this testing strategy. Future research should investigate the fixed and variable costs of pooled testing, as it requires a specific laboratory configuration. Finally, to better understand the impact of pooling, larger studies are needed to develop better sensitivity models as a function of pool size.

ACKNOWLEDGMENTS

We would like to acknowledge Kelsey Oyong, an epidemiologist at the Los Angeles County Department of Public Health who provided us with data on SNFs from the National Healthcare Safety Network. We thank the California Center for Population Research at UCLA (CCPR) for support. CCPR receives population research infrastructure funding (P2C-HD041022) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). This work was partially supported by NIH National Center for Advancing Translational Sciences (NCATS) UCLA CTSI grant numbers UL1TR001881 and TL1TR001883 and the UCLA David Geffen School of Medicine (DGSOM) – Broad Stem Cell Research Center (BSCRC) COVID-19 Research Award OCRC #20-44. Contents are the authors' sole responsibility and do not represent official views of NIH, Los Angeles County Department of Public Health, or any other agency.

AUTHOR CONTRIBUTIONS

Nianogo participated in the study conception, design and analysis, and finalized the complete first draft of the article. Emeruwa wrote the introduction of the manuscript and revised the manuscript. Gounder, Manuel, Arah, Inkelas, and Kuo conceived and supervised the design and analysis, and critically reviewed and revised the manuscript. All authors provided critical input and insights into the development and writing of the article and approved the final manuscript as submitted.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.27054

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are publicly available at https://data.cms.gov/. An online calculator with graphing capabilities which gives immediate information for pooled testing can be found at: https://simrock.shinyapps.io/pool_testing/.

ORCID

Roch A. Nianogo https://orcid.org/0000-0001-5932-6169
Prabhu Gounder https://orcid.org/0000-0002-8600-0348
Vladimir Manuel https://orcid.org/0000-0001-8101-5885
Nathaniel W. Anderson https://orcid.org/0000-0002-4305-2447
Tony Kuo https://orcid.org/0000-0002-4120-8559
Moira Inkelas https://orcid.org/0000-0003-1963-2430
Onyebuchi A. Arah https://orcid.org/0000-0002-9067-1697

REFERENCES

1. White House. Proclamation 9994 - Proclamation on Declaring a National Emergency Concerning the Novel Coronavirus Disease (COVID-19) Outbreak | The White House. https://www.whitehouse.gov/presidential-actions/proclamation-declaring-national-emergency-concerning-novel-coronavirus-disease-covid-19-outbreak/. Accessed June 30, 2020.
2. Governor Newsom Declares State of Emergency to Help State Prepare for Broader Spread of COVID-19 | California Governor. https://www.gov.ca.gov/2020/03/04/governor-newsom-declares-state-of-emergency-to-help-state-prepare-for-broader-spread-of-covid-19/. Accessed June 30, 2020.
3. CDC. Performing Facility-wide SARS-CoV-2 Testing in Nursing Homes. https://www.cdc.gov/coronavirus/2019-ncov/hcp/nursing-homes-facility-wide-testing.html. Accessed June 30, 2020.
4. California Department of Public Health. Coronavirus Disease 2019 (COVID-19) Mitigation Plan Recommendations for Testing of Health Care Personnel (HCP) and Residents at Skilled Nursing Facilities (SNF). https://www.cdph.ca.gov/Programs/CHCQ/LCP/Pages/AFL-20-53.aspx. Accessed June 30, 2020.
5. Los Angeles County Department of Public Health. Guidelines for Preventing and Managing COVID-19 in Skilled Nursing Facilities. http://ph.lacounty.gov/acd/ncorona2019/snf.htm. Accessed August 18, 2020.
6. Testing Guidelines for Nursing Homes | CDC. https://www.cdc.gov/coronavirus/2019-ncov/hcp/nursing-homes-testing.html. Accessed August 18, 2020.
7. Coronavirus (COVID-19) Update: FDA Issues First Emergency Authorization for Sample Pooling in Diagnostic Testing | FDA. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-first-emergency-authorization-sample-pooling-diagnostic. Accessed August 18, 2020.
8. President Donald J Trump and His Administration Have Created The Best Covid-19 Testing System In The World | The White House. https://www.whitehouse.gov/briefings-statements/president-donald-j-trump-administration-created-best-covid-19-testing-system-world/. Accessed September 12, 2020.
9. Dorfman R. The detection of defective members of large populations. *Ann. Math. Stat.* 1943;14(4):436-440.
10. 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;153:715-718.
11. Weinberg CR. Making the best use of test kits for COVID-19. *Am. J. Epidemiol.* 2020;189(5):363-364.
12. Wang D, McMahan CS, Tebbs JM, Bilder CR. Group testing case identification with biomarker information. *Comput. Stat. Data Anal.* 2018;122:156-166.
13. McMahan CS, Tebbs JM, Bilder CR. Regression models for group testing data with pool dilution effects. *Biostatistics.* 2013;14(2):284-298.
14. Wang D, Mcmahan CS, Gallagher CM. A general regression framework for group testing data, which incorporates pool dilution effects. *Stat. Med.* 2015;34(27):3606-3621.
15. Cherif A, Grobe N, Wang X, Kotanko P. Simulation of pool testing to identify patients with coronavirus disease 2019 under conditions of limited test availability. *JAMA Netw. Open.* 2020;3(6):e2013075.
16. Bateman AC, Mueller S, Guenther K, Shult P. Assessing the dilution effect of specimen pooling on the sensitivity of SARS-CoV-2 PCR tests. *J. Med. Virol.* 2020;93:1-5.
17. McMichael TM, Currie DW, Clark S, et al. Epidemiology of Covid-19 in a long-term care facility in King County, Washington. *N. Engl. J. Med.* 2020;382(21):2005-2011.
18. Department of Public Health - Acute Communicable Disease Control. http://publichealth.lacounty.gov/acd/procs/b73/B73Index.htm. Accessed June 30, 2020.
19. COVID-19 Nursing Home Dataset | Data.CMS.gov. https://data.cms.gov/Special-Programs-Initiatives-COVID-19-Nursing-Home/COVID-19-Nursing-Home-Dataset/s2uc-8wxp. Accessed August 18, 2020.
20. Aragón-Caqueo D, Fernández-Salinas J, Laroze D. Optimization of group size in pool testing strategy for SARS-CoV-2: A simple mathematical model. *J. Med. Virol.* 2020;92:1988-1994.
21. LA County COVID-19 Vaccine - LA County Department of Public Health. http://publichealth.lacounty.gov/media/Coronavirus/vaccine/index.htm. Accessed January 17, 2021.
22. Coronavirus: What went wrong at Germany's Gütersloh meat factory? - BBC News. https://www.bbc.com/news/world-europe-53177628. Accessed June 30, 2020.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Nianogo RA, Emeruwa IO, Gounder P, et al. Optimal uses of pooled testing for COVID-19 incorporating imperfect test performance and pool dilution effect: An application to congregate settings in Los Angeles County. *J Med Virol.* 2021;1-9. https://doi.org/10.1002/jmv.27054