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RESEARCH ARTICLE

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A reduction of the number of assays and turnaround time by optimizing polymerase chain reaction (PCR) pooled testing for SARS-CoV-2

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Abstract

Early detection of the severe acute respiratory syndrome coronavirus 2 infection can decrease the spread of the disease and provide therapeutic options promptly in affected individuals. However, the diagnosis by reverse-transcription polymerase chain reaction is costly and time-consuming. Several methods of group testing have been developed to overcome this problem. The proposed strategy offers optimization of group testing according to the available resources by decreasing not only the number of the assays but also the turnaround time. The initial classification of the samples would be done according to the intention of testing defined as diagnostic or screening/surveillance, achieving the best possible homogeneity. The proposed stratification of pooling is based on branching (divisions) and depth (levels of re-pooling) of the original group in association with the estimated probability of a positive sample. The dilutional effect of the grouped samples has also been considered. The margins of minimum and maximum conservation of assays of pooled specimens are calculated and the optimum strategy can be selected in association with the probability of positive samples in the original group. This algorithm intends to be a useful tool for group testing offering a choice of strategies according to the requirements.

KEYWORDS

COVID-19, group testing, pooled analysis, RT-PCR, sample pooling, SARS-CoV-2, sensitivity, turnaround time

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly expanded around the globe. The confirmed fatality ratio of coronavirus disease 2019 (COVID-19) is ranging from 0.1% to 25% in different countries.[1] Even in the era of COVID-19 vaccines, the early identification and isolation of affected individuals is one of the most important factors for the protection of vulnerable people and the reduction of the disease spread. An analysis comparing the mortality rate and the testing performed in several countries has shown a negative correlation between those two parameters.[2]

Since the start of the outbreak, a variety of testing assays have been developed,[3,4] such as reverse-transcription polymerase chain reaction (RT-PCR) for SARS-COV-2, antigen testing, and serology testing.[5] However, RT-PCR remains the gold standard in detection of SARS-CoV-2.[6]

Numerous RT-PCR assays have been designed aiming to maximize the sensitivity and specificity as well as to shorten the turnaround time and to maximize the number of assessed

samples. To be able to handle a high volume of specimens in a limited time, high throughput systems have been developed.[7]

However, testing for SARS-CoV-2 could be a challenge[8] as it requires a significant number of resources regarding testing facilities, personnel, and testing availability. The cost of testing is a significant constraining factor. Several countries have experienced a shortage of testing capacity. Performing individual testing is time-consuming and it also requires a huge number of resources. For this reason, assays examining samples in groups have been developed, to tackle this issue. The first pooling sample method was developed in 1943 by Dorfan, who used pooled blood samples to test an antigen for syphilis.[9] This technique has been applied for diagnoses of several infectious agents such as malaria, HIV, hepatitis B, and hepatitis C,[10-12] since then. Various methods have been recommended regarding the grouping of the samples, taking into consideration different factors such as the available resources and the turnaround time of the assay.[13] Additionally, important factors concerning the efficacy of the assays such as the sensitivity, the specificity, the limit of detection,[14] and the prevalence of the disease in the area have to be considered.

An important factor in the evaluation of laboratory efficiency is the turnaround time as reported by Lu et al.[15,16] and Hawkins.[16] Delay in turnaround time is an indication of lab performance and has adverse clinical consequences. Diagnostic delays of SARS-CoV-2 infection could result in therapeutic delays of symptomatic patients and could prevent the isolation of asymptomatic individuals.

A significant step of the process of pooling assays is to determine the stratification of grouping of the initial samples. In this study, we introduce a method of RT-PCR testing for SARS-Cov-2 by sample pooling, and we suggest a flexible multi-way process of re-pooling based on the probability of SARS-CoV-2 positivity. We subsequently report the maximum and minimum benefits of each stratified method. The optimum process can be selected based on the available resources and the needs. The final goal is to reduce the cost and the turnaround time.

2 | MATERIALS AND METHODS

An algorithm was constructed for the optimization of testing groups of samples for SARS-CoV-2 infection (Figure 1). The algorithm is based on the separation of the specimens in groups whose number depends on the expected ratio of positive samples. The probability of positive samples in the original group will be estimated according to the intention of testing, which may be characterized as either diagnostic or screening/surveillance.

To construct our algorithm, we make the following fundamental assumption about the nature of a COVID test; one can achieve arbitrarily high sensitivity for a diluted sample by repeatedly performing the test on the same sample. This is true, for example, if the sensitivity of different tests on the same sample represents independent stochastic variables.

Before analyzing the final proposed algorithm, we describe an auxiliary algorithm, similar to the one known in computer science as "binary search." Consider the contrived scenario of searching for the single positive sample among 2^k samples. Firstly, we pool the samples by pairing them, creating pools of 2 samples (2-pools). We then pair the 2-pools into 4-pools, then the 4-pools into 8-pools, and so on until we are finally left with a pair of 2^{k-1} pools, which we combine into a final 2^k pool containing all the samples. During the first step, we consider the 2^k pool for testing (pool under consideration-PUC). The dilutional effect in the pooled sample will result in a lower sensitivity of the assay. Following the aforementioned testing method, we test the pooled samples repeatedly in replicates until the sensitivity is high enough that we are confident in a possibly negative result. In case of such a negative result, we declare the entire pool as negative. If the result is positive, on the other hand, for the second step of the algorithm the two 2^{k-1} pools comprising the 2^k pool

$$C_{N^+,N,b}(0) = 1$$

$$C_{N^+,N,b}(i) = b\,C^+_{N^+,b}(i-1)$$

$$T(S) = \begin{cases} 1 & S < 16 \\ 2 & S < 64 \\ 3 & otherwise \end{cases}$$

$$T^{\#}_{N,b}(N^+) = \sum_{i \in [0, log_b(N))} T(C_{N^+,b}(i))$$

$$t_{N,b}(p) = \frac{1}{N} \sum_{N^+ \in [0,N]} \binom{N}{N^+} p^{N^+} (1-p)^{N-N^+} T^{\#}_{N,b}(N^+)$$

$$max\left\{C^+_{N^+,b}(i)\right\} = min\left\{N^+, \frac{N}{b^i}\right\}$$

$$min\left\{C^+_{N^+,b}(i)\right\} = \left\lceil \frac{N^+}{\frac{N}{b^i}} \right\rceil$$

FIGURE 1 The proposed algorithm. Any symbol A + superscript means "the samples of A that test positive." N, the population to be tested; b, the branching factor of the algorithm; PUC, pool under consideration; $C_{N,N+,b}$, the number of pools in PUC at step I; $T(S)$, the expected number of replicates required to test a pool of size S; $T^{\#}_{N,b}(N^+)$, the total number of tests required using our method to find the N^+ positives in a population of N; $t_{N,b}(p)$, the expected ratio of tests performed with our proposed method and by testing each sample individually

are the PUC. We test one of them in replicates. If the result is positive, we deduce that the positive sample exists in the pool we have just tested, otherwise, it exists in the other 2^{k-1} pool. Following the same procedure, the size of the pools we test decreases until we reach a pool containing only one sample which must be the positive sample. We perform $k+1$ steps in total by this method. Assuming that k is small enough that one replicate offers acceptable efficiency for a 2^k pool (as explained in the discussion, $k < 5$ for our purposes, $2^4 = 16$ samples), we perform one test per step. In a group with more than 16 samples, additional tests are performed in replicates to increase the sensitivity. Therefore, via this method, we can find one positive sample in a population of size N performing $\log_2(N)+1$ tests. For example, from a population of $2^5 = 32$ samples, we can find one positive sample by performing only six tests.

In each step of the proposed algorithm, we test the entire PUC. Each new PUC consists of the sub-pools of all the positive pools in the previous PUC. The negative pools and sub-pools are excluded.

Finally, each constructed pool in the auxiliary algorithm is a 2^i pool (where i is a natural number) and has exactly 2 sub-pools. In the proposed algorithm, we generalize this approach by constructing each pool from b sub-pools. As a result, all pools contain b^i samples. For an instance of the algorithm where the largest pool is of size b^k the parameters b and k are called "branching" and "depth," respectively. Based on these parameters the samples are separated into groups of size b^k and the algorithm is then applied to each group.

3 | RESULTS

Table 1 demonstrates the maximum and minimum percentage of expected assays gained by performing nine different stratifications (combinations of branching and depth) in several estimated COVID-19 prevalences, depending on how the positive samples are

TABLE 1 Demonstration of maximum and minimum gained assays of 9 different stratifications based on the COVID-19 prevalence. Min-t: the minimum test required, Max grp: the size of the group samples, Max Tests Gained: the maximum percentage of tests gained, Min Tests Gained: the minimum percentage of tests gained. Positivity: the estimated rate of test positivity

	Branching	**2**	**2**	**2**	**3**	**3**	**3**	**4**	**4**	**4**
	Depth	**3**	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**
	Max grp	4	8	16	9	27	81	16	64	256
Positivity	Min *t*	1	1	1	1	2	2	1	2	2
1%	Max Tests Gained	71%	82%	86%	83%	85%	87%	86%	88%	92%
	Min Tests Gained	71%	82%	86%	83%	84	84%	86%	86%	85%
5%	Max Tests Gained	56%	62%	65%	64%	67%	77%	66%	77%	87%
	Min Tests Gained	56%	59%	57%	62%	55%	50%	60%	53%	50%
10%	Max Tests Gained	40%	44%	50%	48%	58%	70%	53%	72%	81%
	Min Tests Gained	38%	34%	28%	39%	27%	22%	34%	27%	25%
20%	Max Tests Gained	15%	19%	32%	28%	46%	57%	40%	61%	68%
	Min Tests Gained	7%	−6%	−17%	4%	−10%	−14%	−3%	−13%	−15%

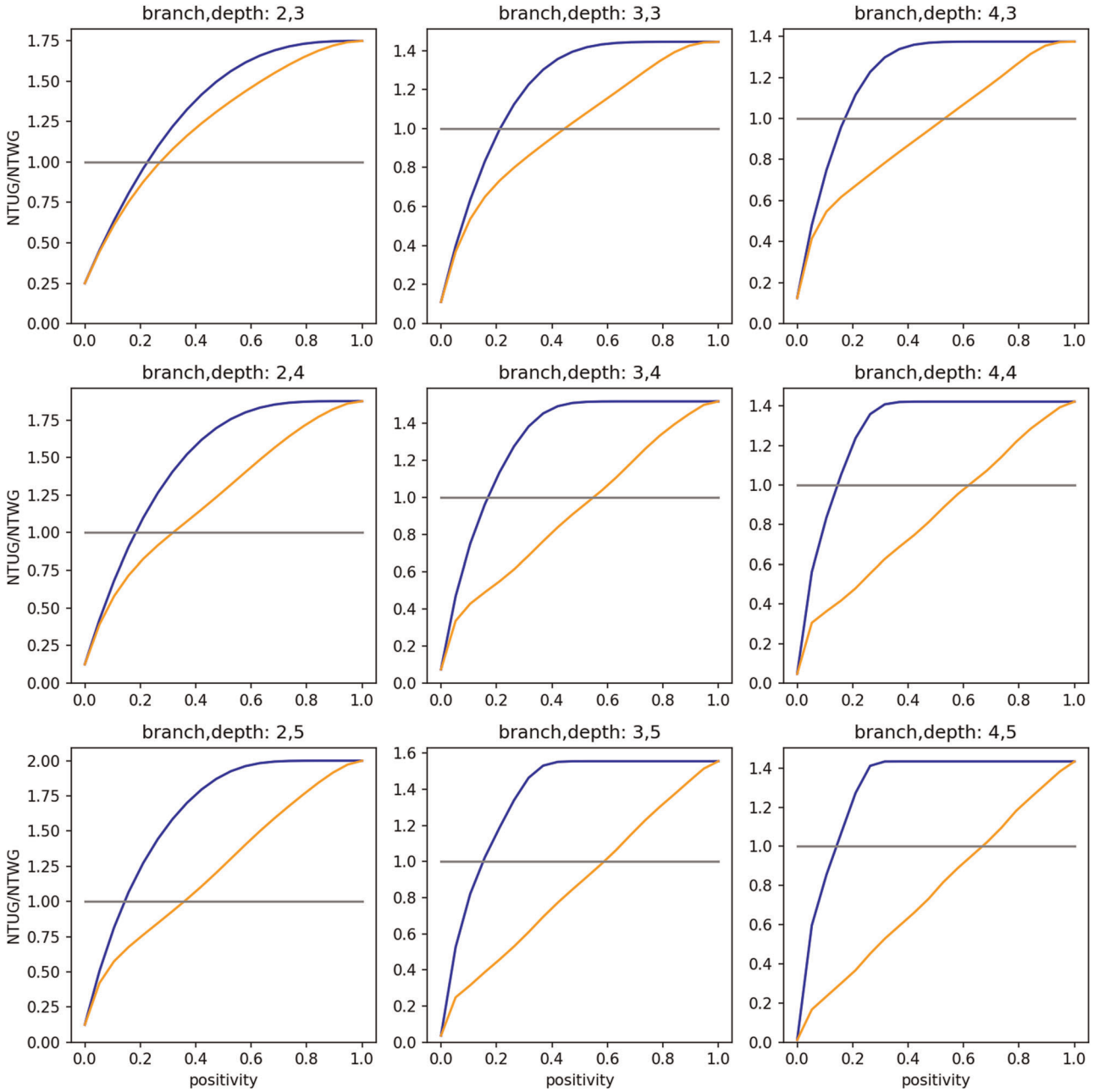

FIGURE 2 Graphic illustration of the ratio of the number of tests using grouping (NTUG) over the number of tests without grouping (NTWG) (NTUG/NTWG) required to identify all the positive samples in different combinations of branching and depth. The vertical axis shows the ratio NTUG/NTWG. The horizontal axis shows the probability of positive samples (percentage of positivity). The blue line shows the maximum possible ratio NTUG/NTWG versus the percentage of positivity, which occurs in the case that the samples are distributed evenly among the groups (worst possible scenario). The orange line shows the same ratio obtained when the samples are distributed with the most favorable distribution (positive samples are concentrated leaving most groups free from positive samples-best possible scenario). The gray line shows the graphic representation of the above parameters when each sample is tested individually (replacing NTUG by NTWG in the estimated ratio)

distributed among the pools. The graphic demonstrations of those nine specific stratifications are shown in Figure 2. The two plots display the maximum and minimum ratios of the number of tests using grouping (NTUG) over the number of tests without grouping (NTWG) (NTUG/NTWG) with respect to the COVID-19 prevalence rate.

These definitions along with the corresponding process are described pictorially in Figure 3A for a particular case of branching = 2, depth = 4, and size of sample = 8. The initial number of samples is divided into groups of 8 and the first assay is performed on these groups. Subsequently, the groups are divided by 2 (branching = 2) into groups of 4 samples each. Assays are performed in each group.

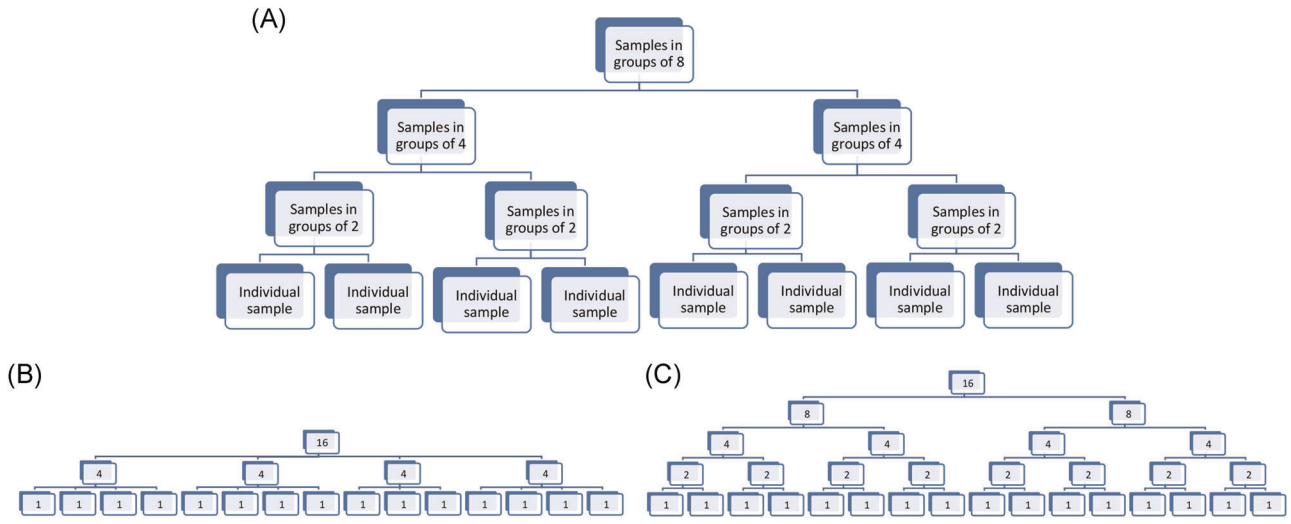

FIGURE 3 (A) Schematic demonstration of branching = 2 and depth = 4 in a group of eight samples and schematic demonstration of comparing two pooling methods with the maximum sample size of 16. (B) Pooling method of branching = 4, depth = 3 and (C) pooling method of branching = 2, depth = 5

This is the first iteration. During the second iteration the sub-groups are divided by 2 into groups of 2 samples each and assays are performed in each sub-group. Finally, on the third iteration, the sub-groups are divided by 2 in individual samples and assays are performed.

The depth (number of iterations) corresponds to the number of times the branching (division) is made. A schematic comparison of different branching and depth is demonstrated in Figure 3B,C. We initially create groups of 16 samples each ($s = 16$). The testing of these groups may be carried out in two ways. We can either follow the stratification of branching $b = 2$ and depth $d = 5$ ($s = 2^4$) or that of branching $b = 4$ and depth, $d = 3$ such that $s = 4^2$. Assuming that the positivity is 1%, both methods offer a minimum reduction of assays by 86% compared to individual testing as shown in Table 1. However, the difference in depth affects the turnaround time of the assay.

Figure 2 shows that the maximum performed assays (blue line), which is the worst-case scenario finally reaching a plateau. The ratio NTUG/NTWG corresponding to the plateau depends on the branching number. Most grouping strategies give NTUG/NTWG > 1 in the worst possible scenario when the positivity reaches 20% This implies that when the probability of a positive sample is above 20%, testing each individual sample is more efficient than grouping. However, grouping is beneficial in two occasions: branching = 2/depth = 3 and branching = 3/depth = 3 in a prevalence rate of 20% (Table 1).

The optimum grouping strategy depends on the percentage of positivity. For example, according to Figure 2, in the case of 10% positivity, the optimum grouping occurs with branching = 3 and depth = 3. This results in a group of nine samples ($s = 3^2$). The minimum and maximum percentages of gained assays are 39% and 48%, respectively (Table 1). However, when the positivity rate is 20%, this type of grouping results in only 4% minimum and 28% maximum of gained assays (Table 1). The same stratification seems to be optimum for 5% positivity. To demonstrate our method more clearly, we illustrate an example in Figure 4. Groups shown in red represent positive samples.

In the case of 1% prevalence, two different strategies (branching = 2/depth = 5 and branching = 4/depth = 3) offer the same beneficial results with an 86% gain of the assays. However,

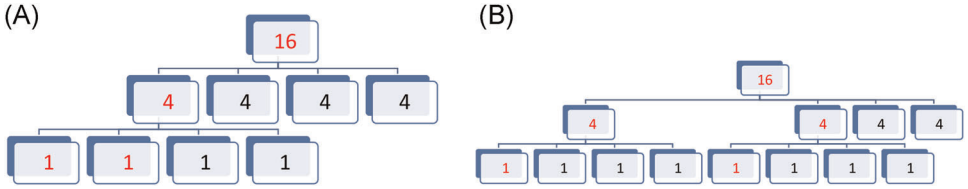

FIGURE 4 Schematic demonstration of the two cases. A group of 16 samples with two samples positive (probability 12.5%) is analyzed with branching = 4 and depth = 3. Case A: grouping with the best possible distribution of positive samples, a total of 9 assays were performed, NTUG/NTWG = 9/16 (0.56). Case B: grouping the worst possible distribution of positive samples, a total of 13 assays were performed, NTUG/NTWG = 13/16 (0.81). NTUG/NTWG, The ratio of the number of tests using grouping (NTUG) over the number of tests without grouping (NTWG). Groups shown in red represent positive samples

the difference in depth has a significant impact on the turnaround time of the reported results. The lower value of depth decreases the turnaround time of the assay.

When the values of the minimum gained assays approach those of the maximum gained assays, then the number of required tests is more predictable. However, this is mostly true in the case of a low positivity rate. As the prevalence of the disease increases, the discrepancy in the values between the maximum and minimum gained assays increases as well. Higher values of branching also increase this discrepancy but in some cases, they offer a more beneficial outcome. Higher values of depth augment only the minimum reduction of the assays (the best possible scenario). These findings are shown in Table 1.

4 | DISCUSSION

RT-PCR has been used as the gold standard in the diagnosis of COVID-19. However, the assay is costly and time consuming. For this reason, pooled assays have been introduced for the testing of asymptomatic individuals in areas with a low prevalence of the disease.[17] These assays are advantageous compared to individual testing as they are not as time-consuming and require a smaller amount of reagents.[18] Both factors are extremely important in case of outbreaks.

In this study, we propose a strategy of RT-PCR grouping assays with flexible stratification, based on the rate of SARS-CoV-2 positivity in the testing specimens. In each case, the lab personnel can balance the importance of time versus cost and select the optimum method regarding the number of branches and depth.

Several theoretical approaches to pooling have been published.[19,20] However, none of the current approaches offers the margins of maximum and minimum reduction of the performed assays following different strategies of pooling in a given disease prevalence. Our method offers a flexible stratification of pooling. The optimum strategy may be selected based on the expected rate of positivity and time constraints. Turnaround time can be shortened by decreasing the depth as shown in Figure 3B,C in cases when the assays can be performed in parallel.

The original specimens can be divided into two categories to achieve the maximum homogeneity of the original group. The division of specimens may be carried out based on the intention of testing, which may include diagnostic or screening/surveillance purposes. The optimum method of pooling may be selected according to the probability of a positive sample existing in the group and time constraints. Based on our algorithm, when the percentage of positivity in the original specimens is high, the most favorable method is associated with smaller group sizes. Groups consisting of fewer samples are probably more homogeneous. However, when the positivity reaches 20% the pooling method is not advantageous compared to individual tests in most cases because the ratio NTUG/NTWG reaches a plateau. Nevertheless, for branching = 2, depth = 3, and branching = 3, depth = 3, a maximum reduction of 7% and 4%, respectively can be achieved by the pooling method. A positivity above 20% is mostly related to tests performed for diagnostic purposes as the prevalence of COVID-19 in several parts of the world is much less than 20%.

Pools consisting of a different number of samples have been suggested by several authors as an optimum stratification. Pikovski et al.[21] stated that the optimal pool size is 4 when the prevalence of the disease is 0%–30% Additionally, templates have been published by the Food and Drug Administration (FDA) for EUA-approved RT-PCR assays, recommending pools of five samples when the prevalence rate is 5-6%.[22] Pools comprised of five samples have been considered optimal by other authors as well.[18] Our method gives optimum results with a minimum 62% reduction of assays in the case of 5% prevalence of COVID-19, with 9 samples per group (branching = 3 and depth = 3). However, for smaller group sizes, a stratification with 4 samples per group, (branching = 2/depth = 3) reduces the original number of assays by 56%. The major advantage in pools of 4 (branching = 2, depth = 3) is that the results are more predictable as shown in Figure 2, where the values of the minimum and maximum gained assays approach each other. It is also noteworthy that these values approach each other, in case of low positivity rates, offering increased predictability of the reduction rate (Figure 2). In the case that the prevalence is 1%, the more predictable results occur in the group size of 16 with branching = 2, depth = 5 and branching = 4, depth = 3 producing an 86% reduction of the assays in both cases. However, the different depths offer different turnaround times. Therefore, the optimal strategy should be selected based on the desired turnaround time. If faster result acquisition is required, implementation of fewer iterations may be beneficial. In contrast, the reduction percentage is less predictable for higher values of depth and branching as the minimum and maximum expected gains drift further apart.

It is well known that false-negative results can set off a cascade of consequences by inhibiting the isolation of an infected person. One of the major factors affecting the test sensitivity is the dilutional effect of the pooled samples. To mitigate the dilutional effect, we perform the assays in replicates in the case of group size above 16 (2^4) before we declare the pooled samples as negative. This decision is based on the report by Kim et al.[23] who have shown that the pooled positive specimens had 100% sensitivity in pool sizes 2, 4, and 6 and 97%–99% sensitivity in pool sizes 8, 10, and 16. Additionally, Yelin et al.[24] have concluded that the dilutional effect is minimal for groups of 32 samples. They have reported that a single positive sample can be detected in a pooling of 32 samples with false-negative rate of 10%. Several other reports have been published regarding the number of samples per grouping. Ben-Ami et al.[25] reported that the sensitivity of the test does not change in pools of five or eight samples. Deckert et al.[19] selected pool sizes between 5 and 50 by comparing a high-throughput approach with a context-sensitivity method. It has been suggested that the viral load is another important factor affecting the sensitivity of the assay in pooled samples. It has been shown that in each two-fold dilution, the cycle

threshold (C_t) value increased by 1.24.[24] This was possible either when pooling before or after the RNA extraction. Specimens with low viral load ($C_t > 35$) showed 13.3% false-negative results.[26] Another study with pooling size 9–10 has reported a loss of 2.87C_t for E gene, 3.36C_t for RdRP gene, and 2.99C_t for N gene.[27] Pools consisting of many samples may require additional amplification cycles due to lower viral load in the pooled samples.

Although RT-PCR and pooling techniques, which are discussed in this study, are widely used for diagnostic and screening purposes, they impose limitations that should be taken into consideration. RT-PCR testing requires equipment, trained personnel, and reagents which are costly.[28] Regarding the pooling technique, the FDA has reported shortcomings such as the dilution of the pool sample, the variability of results depending on the prevalence of the disease in the community, and difficulty in the collection of adequate specimens.[29] A further limitation of our method is the requirement of pre-assay epidemiological evaluation to classify the specimens as diagnostic or as screening/surveillance. In addition, during the proposed process each sample is evaluated several times. Although this has the beneficial effect of improving the sensitivity of the method, it also increases the risk of intralab errors during labeling and augments the possibility of lab contamination.

In conclusion, this algorithm is intended to be a useful tool for laboratory use according to the available resources and the final targeted benefit. Our method gives the flexibility to organize the turnaround time, the personnel involved, and finally the cost.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHORS CONTRIBUTIONS

Christos Perivolaropoulos and VasilikiVlacha contributed equally to the conception, design, analysis, interpretation, drafting, writing of the manuscript and the final approval of the version submitted

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.26972

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Ceylan Z. Estimation of COVID-19 prevalence in Italy, Spain, and France. *Sci Total Environ*. 2020;729:138817. https://doi.org/10.1016/j.scitotenv.2020.138817
2. Liang L-L, Tseng C-H, Ho HJ, Wu C-Y. COVID-19 mortality is negatively associated with test number and government effectiveness. *Sci Rep*. 2020;10(1):12567. https://doi.org/10.1038/s41598-020-68862-x
3. Chau CH, Strope JD, Figg WD. COVID-19 clinical diagnostics and testing technology. *Pharmacother J Hum Pharmacol Drug Ther*. 2020;40(8):857-868. https://doi.org/10.1002/phar.2439
4. Patel R, Babady E, Theel ES, et al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: value of diagnostic testing for SARS–CoV-2/COVID-19. *mBio*. 2020;11(2), https://doi.org/10.1128/mBio.00722-20
5. Ma H, Zeng W, He H, et al. Serum IgA, IgM, and IgG responses in COVID-19. *Cell Mol Immunol*. 2020;17(7):773-775. https://doi.org/10.1038/s41423-020-0474-z
6. Böger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *Am J Infect Control*. 2021;49(1):21-29. https://doi.org/10.1016/j.ajic.2020.07.011
7. Pfefferle S, Reucher S, Nörz D, Lütgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. *Euro Surveill*. 2020;25(9), 2000152 https://doi.org/10.2807/1560-7917.ES.2020.25.9.2000152
8. Binnicker MJ. Challenges and controversies to testing for COVID-19. Kraft CS, ed. *J Clin Microbiol*. 2020;58(11):e01695-20./jcm/58/11/JCM.01695-20.atom https://doi.org/10.1128/JCM.01695-20
9. Dorfman R. The detection of defective members of large populations. *Ann Math Stat*. 1943;14(4):436-440. https://doi.org/10.1214/aoms/1177731363
10. Taylor SM, Juliano JJ, Trottman PA, et al. High-throughput pooling and real-time PCR-based strategy for malaria detection. *J Clin Microbiol*. 2010;48(2):512-519. https://doi.org/10.1128/JCM.01800-09
11. Emmanuel JC, Bassett MT, Smith HJ, Jacobs JA. Pooling of sera for human immunodeficiency virus (HIV) testing: an economical method for use in developing countries. *J Clin Pathol*. 1988;41(5):582-585. https://doi.org/10.1136/jcp.41.5.582
12. García Z, Taylor L, Ruano A, et al. Evaluation of a pooling method for routine anti-HCV screening of blood donors to lower the cost burden on blood banks in countries under development. *J Med Virol*. 1996;49(3):218-222. https://doi.org/10.1002/(SICI)1096-9071(199607)49:3%3C218::AID-JMV10%3E3.0.CO;2-8
13. Bilder CR, Tebbs JM. Pooled-testing procedures for screening high volume clinical specimens in heterogeneous populations. *Stat Med*. 2012;31(27):3261-3268. https://doi.org/10.1002/sim.5334
14. Arnaout R, Lee RA, Lee GR, et al. SARS-CoV2 testing: the limit of detection matters. *BioRxiv Prepr Serv Biol*. Published online June 4 2020. https://doi.org/10.1101/2020.06.02.131144
15. Lu Y, Leong W, Wei B, et al. An evaluation of laboratory efficiency in Shanghai emergency by turn around times level. *J Clin Lab Anal*. 2015;29(4):334-341. https://doi.org/10.1002/jcla.21775
16. Hawkins RC. Laboratory turnaround time. *Clin Biochem Rev*. 2007;28(4):179-194.
17. Health C for D and R . Pooled Sample Testing and Screening Testing for COVID-19. FDA. Published online August 24, 2020. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/pooled-sample-testing-and-screening-testing-covid-19. Accessed January 3, 2021.
18. 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(6):715-718. https://doi.org/10.1093/ajcp/aqaa064
19. Deckert A, Bärnighausen T, Kyei NN. Simulation of pooled-sample analysis strategies for COVID-19 mass testing. *Bull World Health Organ*. 2020;98(9):590-598. https://doi.org/10.2471/BLT.20.257188

20. Gollier C, Gossner O. Group testing against COVID-19. *Covid Economics*. 2020.1(2):32–42.
21. Pikovski A, Bentele K. Pooling of coronavirus tests under unknown prevalence. *Epidemiol Infect*. 2020:148. https://doi.org/10.1017/S0950268820001752
22. Health C for D and R. In Vitro Diagnostics EUAs. FDA. Published online December 30, 2020. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas. Accessed January 3, 2021.
23. Kim SY, Lee J, Sung H, et al. Pooling upper respiratory specimens for rapid mass screening of COVID-19 by real-time RT-PCR. *Emerg Infect Dis*. 2020;26(10):2469-2472. https://doi.org/10.3201/eid2610.201955
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. https://doi.org/10.1093/cid/ciaa531
25. 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. https://doi.org/10.1016/j.cmi.2020.06.009
26. Wacharapluesadee S, Kaewpom T, Ampoot W, et al. Evaluating the efficiency of specimen pooling for PCR-based detection of COVID-19. *J Med Virol*. 2020;92(10):2193-2199. https://doi.org/10.1002/jmv.26005
27. de Salazar A, Aguilera A, Trastoy R, et al. Sample pooling for SARS-CoV-2 RT-PCR screening. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2020;26(12):1687.e1-1687.e5. https://doi.org/10.1016/j.cmi.2020.09.008
28. Nguyen T, Duong Bang D, Wolff A. 2019 Novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics. *Micromachines*. 2020;11(3):306. https://doi.org/10.3390/mi11030306
29. Policy for Coronavirus Disease. Tests During the Public Health Emergency (Revised)—Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff. 2019. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised

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