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Optimally pooled viral testing

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ABSTRACT

It has long been known that pooling samples may be used to reduce the total number of tests required in order to identify each infected individual in a population. Pooling is most advantageous in populations with low infection (positivity) rates, but is expected to remain better than non-pooled testing in populations with infection rates up to 30%. For populations with infection rates lower than 10%, additional testing efficiency may be realized by performing a second round of pooling to test all the samples in the positive first-round pools. The present predictions are validated by recent COVID-19 (SARS-CoV-2) pooled testing and detection sensitivity measurements performed using non-optimal pool sizes, and quantify the additional improvement in testing efficiency that could have been obtained using optimal pooling. Although large pools are most advantageous for testing populations with very low infection rates, they are predicted to become highly non-optimal with increasing infection rate, while pool sizes smaller than 10 remain near-optimal over a broader range of infection rates.

1. Background

The advantages of pooled testing in applications ranging from disease screening to manufacturing quality assurance have long been appreciated (Dorfman, 1943). Efficiently and practically containing viral outbreaks requires minimizing the total number of tests required to uniquely identify every positive individual. This may be achieved using pooled testing, given a sufficiently sensitive diagnostic test with an acceptably low false-negative detection probability. When applicable, pooled testing a large number N of individuals can be achieved with significantly fewer than N tests, by initially screening pools containing a mixture of samples from n individuals, followed by further testing of only the positive pools to uniquely identify each infected individual. The latter tests may either be carried out by separately testing all individuals in the positive first-round pools or by using a second round of pooling to more efficiently identify each infected individuals in the positive first-round pools. The present results provide optimal first and second round pool size predictions for populations with infection probabilities of $0.001 \leq p < 0.3$ (corresponding to positivity rates between 0.1% and 30%), as well as the range of infection probabilities over which a given fixed pool size remains near-optimal.

The primary aim of this work is to provide practical guidance to SARS-CoV-2 (COVID-19) testing centers regarding the efficient implementation of pooled testing. Specifically, the present predictions (which are most conveniently summarized in Table 1) may be used to guide the selection of an optimal pool size for a population with a given estimated positivity rate and estimate the corresponding maximum number of tests that will be required to identify every infected individual (expresses as a percentage of the total number of tested individuals). Those readers that are primarily interested in clinical applications of the present results (rather than the associated mathematical derivations) may skip the *Methods* Section and focus primarily on Table 1 which contains the optimal first and second round pool size and testing percentage predictions.

The present predictions are obtained assuming that the population of interest has a uniform infection rate, which may be detected with perfect accuracy and specificity. In spite of these idealizations, the practical utility of the predictions are quantitatively validated by recent SARS-CoV-2 pooled testing data (Lohse et al., 2020; Yelin et al., 2020; de Salazar et al., 2020; Hogan et al., 2020; Ben-Ami et al., 2020; Bullard et al., 2020). The results indicate that pooled testing can significantly reduce the number of the SARS-CoV-2 tests required to identify each positive individual, even in populations with infection rates above 10%, although pooled testing is most advantageous in populations with lower infection rates. The field testing validation studies also confirm that the present predictions provide conservative testing efficiency estimates, as non-uniform clustering of infections is predicted to lead to an increase in testing efficiency, above that predicted assuming a uniform infection rate.

The optimal pool size, n, for a population with a given infection probability p was first obtained by Dorfman (1943) (and has since spawned numerous generalizations) (Aldridge et al., 2019; Brault et al., 2020; Ben-Ami et al., 2020). Here Dorfman's results are extended to

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yield practically useful predictions of the range of infection rates over which a given fixed pool size remains nearly optimal, as well as the significant additional efficiency that may be obtainable from using a second round of pooling for populations with $0.001 \leq p < 0.1$ ($0.1\% \leq p\% < 10\%$). For a populations with a very low infection rate of 0.1%, the predicted 1st round optimal pool size is 32. The practicality of using pools this large has recently been demonstrated by showing that a standard RT-qPCR test for SARS-CoV-2 may be used to detect a single positive individual in pools as large as 32, with 90% accuracy (corresponding to a false negative rate of 10%) (Yelin et al., 2020), which is consistent with results reported in a recent article in *Lancet* (Lohse et al., 2020). However, it is also important to note that tests performed using such large pools are only predicted to be beneficial for populations with a very low (and narrow) range of infection rates, and become highly non-optimal for populations with infection rates exceeding 1%. Thus, for example, for infection rate near or exceeding 10%, optimal pooling efficiency requires using pools smaller than 5.

In practice, these predictions may be used by initially choosing a pool size that is optimal for the estimated infection probability in the population of interest, and subsequently adjusting the pool size to better match the actual infection probability. Although pooling incurs delays associated with repeated measurements, those delays are offset by the fact that fewer measurements are required to test a given population. These predictions are expected to be most useful in facilitating large scale screening and continuous testing of populations with low infection probabilities to provide early warning of SARS-CoV-2 resurgence, and thus enhance both public safety and economic productivity.

2. Methods

The binomial distribution yields the following expression for the probability that there will be k infected individuals in a pool of sized n, drawn from a population with an infection probability of p (Wilcox et al., 2013)

$$P(k) = \frac{n! p^k (1-p)^{n-k}}{k!(n-k)!} \tag{1}$$

When $k = 0$ this reduces to the following expression for the fraction of pools that are expected to contain no infected individuals, in keeping with Dorfman's original predictions (Dorfman, 1943)

$$P(0) = (1-p)^n \tag{2}$$

This yields the following expression for the total number of tests N_{tests} required in order to identify every positive individual in a population of size N, when using a pool size of n.

$$N_{tests} = \frac{N}{n} + N\left[1 - (1-p)^n\right] \tag{3}$$

Thus, the predicted average percentage of tests that must be performed in order to identify every infected individual in a population of size N is $T\% = 100 \times (N_{tests}/N)$. In other words, $T\%$ represents the average number of tests required to identify each infected individual in a population of size 100, or equivalently $T\%\times 1000$ is the number of tests required to do so in a population of 100,000.

$$T\% = 100\left[\frac{1}{n} + 1 - (1-p)^n\right] \tag{4}$$

The optimal value of n is that which minimizes $T\%$, and thus may be obtained by finding the roots of the following expression for the partial derivative of $T\%$ with respect to n, for a given value of p.

$$-\frac{1}{100}\left(\frac{\partial T\%}{\partial n}\right)_p = \frac{1}{n^2} + (1-p)^n \ln(1-p) = 0 \tag{5}$$

The above expression may be solved numerically using Newton's method. Alternatively, the optimal pool size may also be obtained iteratively, using an initial guess for the pool size n_0, inserted into the right-hand-side of the following expression, to obtain a better estimate of n (where the "Round" operation rounds the result to the nearest positive integer).

$$n \approx \text{Round}\left\{\left[\ln\left(\frac{1}{1-p}\right)(1-p)^{n_0}\right]^{-1/2}\right\} \tag{6}$$

If n_0 is not very similar to n, then one may set $n_0 = n$ and repeat the process to obtain a better estimate of n. This iterative procedure typically converges within a few cycles (whose convergence can be most accurately quantified by removing the Round operation from the right-hand-side of Eq. (6)). Note that the optimal pool size may also be approximated using $n \approx \text{Round}(1 + 1/\sqrt{p})$ (Finucan, 1964)

The infected individuals in the positive first-round pools may in some cases be more efficiently determined using a second round of pooled testing. The average infection probability p_2 in all the positive first round pools is higher than that in the original population because all the non-infected individuals in the negative first round pools have been removed from the population of second round test samples. Thus, the optimal second round pool size n_2, pertaining to an infection probability of p_2, may be obtained as follows (using Eqs. (5) or (6)).

$$p_2 = \frac{p}{1-(1-p)^n} \tag{7}$$

$$n_2 = n(p_2) \tag{8}$$

The above results imply that employing two rounds of pooled testing is only advantageous for a population with a positivity rate less than 10% ($p \leq 0.1$), as the predicted value of p_2 exceeds 0.3 at higher infection rates. The following equation predicts the total number of tests required to identify every infected individual when using two rounds of optimal pooling.

$$T\%(\text{optimal 2 round total}) = \frac{100}{n} + \left[1-(1-p)^n\right] T\%(p_2, n_2) \tag{9}$$

Note that $100/n$ is the number of pools that were tested in the first round (expressed as a percent of total number of tested individuals N), and $1-(1-p)^n$ is the fraction of positive first round pools, and thus $[1-(1-p)^n]\,T\%(p_2, n_2)$ is the number of tests required to identify all the positive individuals in those pools, where $T\%(p_2, n_2)$ is obtained using Eq. (4) (with $p = p_2$ and $n = n_2$).

More generally, Eqs. (4) and (9) may also be used to obtain predictions pertaining the efficiency of non-optimally pooled tests, for a population with a given average infection probability p, and any chosen values of n and n_2.

3. Results

Table 1 contains optimal pooled testing predictions pertaining to populations with uniform positivity rates ranging from 0.1% to 30%. The 3rd column contains the predicted optimal first-round pool size n (obtained using Eqs. (5) or (6)), and the 4th column contains the resulting first round testing percentage $T\%$ predictions (corresponding to averages over large test populations). The last two columns in Table 1 pertain to predictions obtained when using a second round of pooling to test all the positive first-round pools. For example, testing 100,000 individuals in a population with a positivity rate of 1% is predicted to require only 20,000 tests when using one round of optimal pooling, and the total number of tests may be further reduced to 15,000 when using a second round of optimal pooling. Moreover, the field test validation studies described in the *Discussion and Results* Section imply that these predictions represent upper bound estimates, as a population with a non-uniform infection rate is expected require even fewer tests that the above predictions.

Fig. 1 contains more detailed predictions pertaining to the average number of infected individuals in pools of optimal size, when the overall infection probability ranges from 1% to 30%. Note that

Table 1
Optimally pooled testing predictions.

Infection probability (p)	Positivity rate (p%)	Pool size 1st round (n)	Tests needed 1 round (*T*%)	Pool size 2nd round (n_2)	Tests needed 2 rounds (*T*%)
0.001	0.1	32	6	6	4
0.002	0.2	23	9	5	6
0.003	0.3	19	11	5	8
0.004	0.4	16	12	5	9
0.005	0.5	15	14	4	10
0.006	0.6	13	15	4	12
0.007	0.7	12	16	4	13
0.008	0.8	12	18	4	13
0.009	0.9	11	19	4	15
0.01	1	11	20	4	15
0.02	2	8	27	3	23
0.03	3	6	33	3	30
0.04	4	6	38	3	34
0.05	5	5	43	3	39
0.06	6	5	47	3	43
0.07	7	4	50	3	49
0.08	8	4	53	3	52
0.09	9	4	56	3	55
0.1	10	4	59	3	59
0.15	15	3	72		
0.2	20	3	82		
0.25	25	3	91		
0.3	30	3	99		

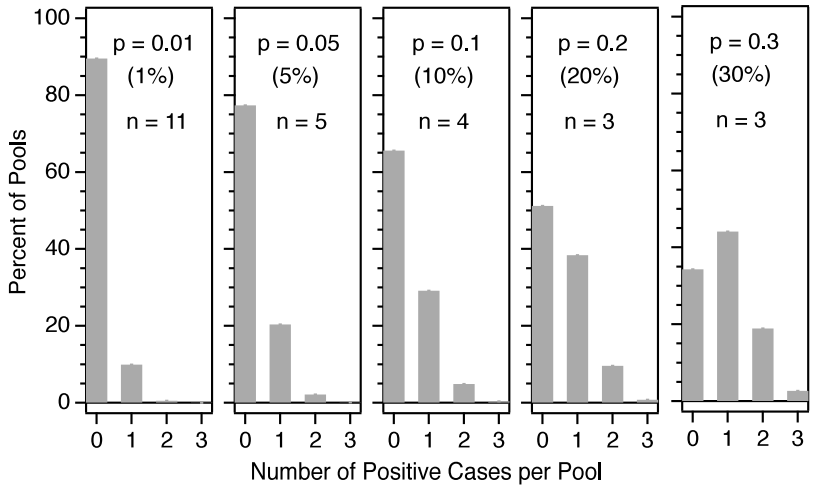

Fig. 1. Predicted number of infected individuals in optimally sized pools obtained from populations with average infection rates ranging from 1% to 30%.

at (and below) an infection probability of 1%, essentially all of the positive pools are predicted to contain only one infected individual. At higher infection probabilities a non-negligible number of positive pools are predicted to contain more than one infected individual, but nevertheless most positive pools are predicted to contain only one infected individual. For example, even in a population with an infection probability of 30%, about 34% of the pools of size 3 are predicted to contain no infected individuals, while 45% contain one, and only 21% contain more than one infected individual. However, at this high rate of infection pool testing is no longer advantageous, relative to exhaustively testing every single individual, as indicated by the 4th column in Table 1, which indicates that an average of 99 tests would have to be performed when optimally pool testing a population of 100 individuals that has an infection rate of 30%.

Figs. 2 and 3 contain graphical predictions pertaining to tests performed using either one or two rounds of optimal pooling, respectively. Fig. 2 shows the resulting optimal first round pool size n (**a**) and testing percentage T% (**b**) predictions. The inset panels in each figure contain an expanded view of the predictions pertaining to populations with infection rates less than 1% ($p \leq 0.01$), and the solid curves are optimal pooled testing predictions. The optimal pool size values shown in Table 1 are obtained by rounding the graphical results to the nearest positive integer. The dotted curves in Fig. 2**b** show the testing efficiency predictions obtained when using various non-optimal pool sizes n_0. These predictions indicate that pool sizes of 5, 6, and 7 are expected to produce nearly optimal testing efficiency in populations with average infection rates of 2%–12%, 1%–8%, and 0.7%–6%, respectively (as determined by requiring that T% remain within 3% of its optimal value). The dotted curves in the inset panel in Fig. 2**b** illustrate the fact that large pool sizes are only expected to be optimal over a very narrow range of infection probabilities, and to rapidly become significantly non-optimal with increasing infection probability.

Fig. 3, as well as the last two columns in Table 1, contain predictions pertaining to the application of two rounds of optimal pooling, performed on the same population of test samples. Specifically, the 5th column in Table 1 contains the optimal second round pool size and the last column contains the predicted average number of tests required to determine all the infected individuals in a population of size 100 when using two rounds of optimal pooling. The second round of optimal pooling is performed by limiting the second round tests to individuals in the positive first round pools.

The solid curves in Fig. 3 are optimal second round pool testing predictions. Fig. 3**a** shows the predicted optimal pool size, n_2, that should

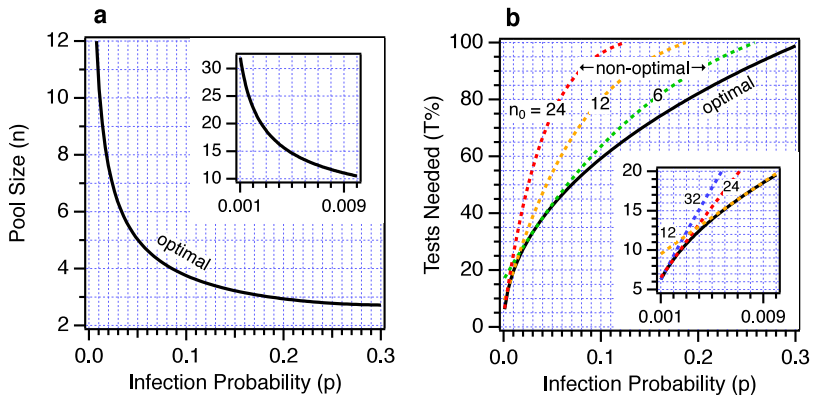

Fig. 2. Optimal pool size **a** and testing percentage **b** predictions obtained when applying a single round of pooled testing. The dashed lines in **b** represent the testing percentages obtained using three different non-optimal pool sizes.

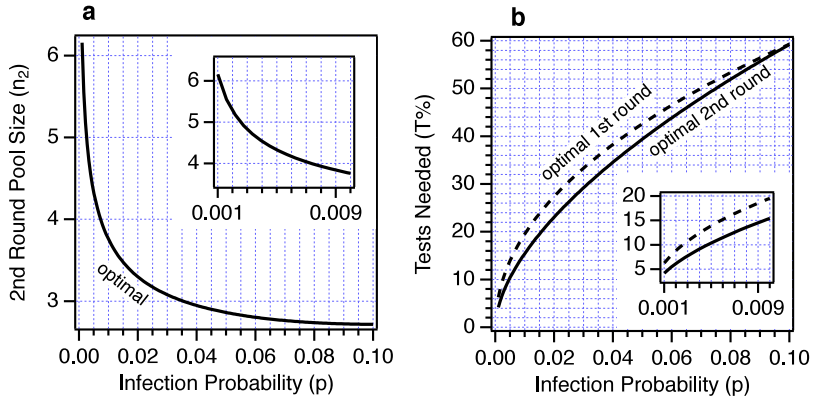

Fig. 3. Predicted optimal pool sizes **a** and testing percentages **b** obtained when applying two rounds of pooled testing. The dashed and solid curves in **b** compare the predicted testing efficiencies obtainable using one or two rounds of optimized pool testing, respectively.

be used in order to efficiently re-test all the samples from the positive first round pools. The dashed and solid curves in Fig. 3**b**, as well as the 4th and 6th columns in Table 1, compare the first and second round optimal testing efficiency predictions. Note that using a second round of pooling is only predicted to be advantageous for populations with positivity rates above 10%, and using two rounds of pooling becomes increasing advantageous as the positivity rate decreases. For example, for a population of 100,000 with a positivity rate of 0.1%, the predicted number of tests decreases from 6000 to 4000 when using one or two rounds of optimal pooling, respectively.

4. Discussion and summary

The primary results of this study are contained in Table 1, which identifies the optimal first and second round pool sizes for a populations with a given positivity rate ($p\%$). The key advantage of pooled testing is the reduced cost (and supplies) required to test a given population. Although pooled testing necessarily introduced time delays associated with repeated testing cycles, it is expected that these delays will be offset by the decreased turn-around time associated with the reduction in the total number of tests required when implementing optimal pooling.

Although the present theoretical prediction have not yet been fully clinically validated, the accuracy of the predictions may nevertheless be critically tested using comparisons with recently reported SARS-CoV-2 pooled test data obtained using non-optimal pool sizes. For example, as recently reported in *Lancet* (Lohse et al., 2020), two rounds of pooled tests for SARS-CoV-2 were performed on 1191 samples with an average infection rate of 1.93%, using 1st and 2nd round pool sizes of 30 and 10, respectively. A total of 267 tests were required in order to identify each of the 23 infected individuals in that population, corresponding to $T\% = 100 \times (267/1191) = 22.4\%$, which is quite close to the predicted value of $T\% = 23.7\%$ (corresponding to 282 tests) obtained using Eq. (9) assuming the same infection rate and pool sizes. Moreover, the present predictions imply that about 15 fewer tests would have been required if more nearly optimal 1st and 2nd round pool sizes of 8 and 3, respectively, had been used to test the same population.

As another example, an early SARS-CoV-2 pool testing study used a pool size of 10 to test 2888 samples obtained from a population with an average infection rate of 0.07% (Hogan et al., 2020). These pooled tests correctly identified the two infected individuals in this population using a total of 312 tests, or T% = 10.8%, which compares very well with T%=10.7% predicted using Eq. (4). Yet another validation of the present predictions is obtained from recent test of 2160 samples from a population with an infection rate of 0.23% using a pool size of 8 (Ben-Ami et al., 2020), in which SARS-CoV-2 five infected individuals were identified using at total of 311 tests, or T% = 14.4%, which compares very favorably with T% = 14.3% predicted using Eq. (4). In both of the above examples the present predictions imply that a substantial additional gain in pooled testing efficiency could have been obtained by using a more nearly optimal pooling strategy. Specifically, if the first population (Hogan et al., 2020) were tested using optimal 1st and 2nd round pool sizes of 38 and 7, respectively, the required number of tests is predicted to decrease $T\%$ to 3% (from ~11%), and in the second population (Ben-Ami et al., 2020) the use of optimal 1st and 2nd round pool sizes of 21 and 5, respectively, is predicted to decrease $T\%$ to 7% (from ~ 14%).

Even more interestingly, another SARS-CoV-2 study reported pooled testing results for 2519 samples performed using a pool size of $n = 10$ or 11 (de Salazar et al., 2020). A total of 1243 tests were required to

identify every one of the 241 positive individuals in this population with an average infection rate of $p = 241/2519 = 0.096$ (9.6%) (de Salazar et al., 2020). In this case Eq. (4) predicts that a somewhat larger number of $2519 \times T\%(0.096, 10)/100 = 1853$ tests should have been required to identify every infected sample. The fact that only 1243 rather than 1853 tests were required suggests that a significant number of the positive samples were clustered together in the 99 positive pools, rather than more uniformly distributed over the predicted number of approximately 160 positive pools. Thus, any non-uniformity in the positivity rate (in a population with a given average positivity rate) is expected to decrease the required number of tests below the predictions shown in Table 1.

In a population with an infection rate of 0.1% the predicted optimal pool size is 32, which is consistent with recently reported SARS-CoV-2 testing sensitivities achievable using a standard RT-qPCR test (Yelin et al., 2020; Lohse et al., 2020). In practice, an upper bound to the pool size is dictated by the cycle threshold (Ct) pertaining to samples in the population of interest and the particular RT-qPCR test equipment and protocols (Lohse et al., 2020; Bullard et al., 2020; Ben-Ami et al., 2020). Importantly, a recent clinical study concluded that infective SARS-CoV samples tend to have $Ct < 24$ (Bullard et al., 2020), while another study indicated that even when diluted in pools of size 30 the Ct of all SARS-CoV-2 positive pools remained below 30 Lohse et al. (2020). Thus, again suggesting that pools as large 30 may be used to successfully detect all individuals with significant SARS-CoV-2 viral loads and infectivity. More generally, once a maximum pool size is established, then that maximum pool size should be used instead of any larger optimal pool size appearing in Table 1. Moreover, when using a non-optimal first-round pool size, then the optimal second round pool size should be re-calculated using Eqs. (7) and (8). However, when the optimal pool size listed in Table 1 is less than or equal to the maximum pool size, then pooled testing efficiency is expected to improve when using the smaller pool sizes listed in Table 1.

Although it is often assumed that pooled testing is only useful for populations with infection rates below about 5%, the present results indicate that even when the infection rate is 10%, pooling can be used to decrease the number of required tests by approximately 40% (relative to individual testing), and a single round of pooled testing remains advantageous for positivity rates up to 30%, as long as the associated pool size is sufficiently small. Moreover, any variability of infection rates within a population with a given average infection rate is expected to improve, rather than degrade, the efficiency of pooled testing, as clustering of positive samples in fewer positive pools will decease the number of pools that need to be individually tested.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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