Pooled Sample Testing for SARS-CoV-2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resultant coronavirus disease (COVID-19) are spreading rapidly across the globe. However, testing for the virus remains limited due to resource constraints. Pooled sample testing provides one approach to significantly increase testing capacity. In its simplest and standard form, samples from multiple individuals are pooled together and tested. If a pooled sample is negative, all individuals in the pooled sample are deemed negative. If the pooled sample is positive, the individual samples from the pool are then tested. Consider a simple example with a population of 50 individuals, one of whom is infected. All 50 individuals can be tested individually or 5 pooled samples of 10 individuals can be tested. One of the pooled samples will be positive and the 10 individual samples in the positive pool can then be tested. This approach uses 15 tests in total compared to the 50 used in individual testing. Pooled testing was introduced by Dorfman in the 1940s to screen syphilitic men in the US military [1]. Since then, it has been employed numerous times in the medical field for infections including influenza [2], chlamydia [3], and malaria [4]. Notably, it was used in the early stages of the HIV pandemic when polymerase chain reaction (PCR) test costs were high [5]. Several countries, including the US [6], Israel [7], and Germany [8], have already demonstrated the efficacy of pooled testing for SARS-CoV-2 and have implemented pooled testing methodologies to increase capacity. This note briefly covers the some of the basic statistical details of pooled testing and relevant medical literature.

Statistical methods

The Dorfman approach, described in the example above, is a common and simple pooled testing and retesting procedure used in medicine. N individual samples are pooled into N/n pools of size n. These pools are tested and samples from positive pools are then tested individually. This approach assumes away potential false positives/negatives. For a homogeneous population with infection probability p, the expected number of tests T used is simply

$$\mathrm{E}[T] = \frac{N}{n} + nq\frac{N}{n} \tag{1}$$

where $q = 1 - (1-p)^n$ is the probability at least one sample in a pool of size n is infected. Decomposing this expectation, we have N/n pools to test in the first round and then n times the number of positive pools to test in the second round. The number of positive pools from the first round is a random variable and is distributed Binomial(N/n, q) with expectation $q(N/n)$. For a fixed E[T] and p, we can use equation (1) to solve for the pool size n that maximizes the number of individuals tested N. The optimal group size to maximize the number of individuals tested given an expected number of tests is

$$n^* = 2\,\frac{W\big(-\frac{1}{2}\sqrt{-\ln(1-p)}\,\big)}{\ln(1-p)} \tag{2}$$

where $W(x)$ is the Lambert W function (product log). Interestingly, n^* depends only on p, and not on E[T]. Figure 1A displays optimal pool size as a function of p. Small values of p imply large values of n^*. In this case, the probability that a large pool tests negative is large. Therefore, a large n^* can be used to classify a large number of individuals as negative with one single test. For large values of p, the probability that a large pool tests positive and all the individual in the pool require retesting is large, so n^* is small. Figure 1B reports the number of tested individuals as a function of n for E[T] = 1500 and two values of p. For $p = 0.02$, a pool size of roughly 8 allows for roughly 5500 individuals to be tested using only 1500 tests in expectation. For $p = 0.002$, a pool size of roughly 23 allows for roughly 17,000 individuals to be tested. As implied by Figure 1, the benefits of pooled testing are greatest when prevalence, p, is low.

If the number of available tests is limited and we use the Dorfman approach to determine both pool size and total sample size, there is a significant probability that we run out of tests and do not learn test results for all the individuals in the sample. This could be a concern

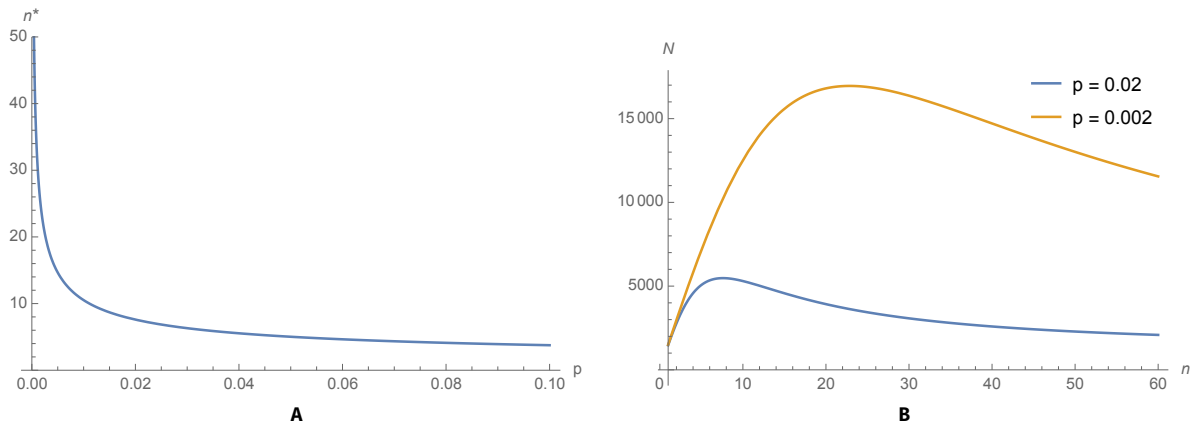

Figure 1: **A)** Optimal pool size n^* for Dorfman testing as a function of prevalence. **B)** The number of individuals N that can be tested as a function of pool size n for 1500 tests in expectation and two values of p.

if there is some expectation that test results will be available for all the individuals in the sample. It could also complicate the analysis of the data if individuals in positive pools are left untested. It is, however, easy to calculate the probability of running out of tests under the Dorfman rule. Suppose that the number of available tests is 1500. Let B be the number of positive pooled tests. Because B is Binomial$(N/n,\, q)$, we obtain $T = (N/n) + nB$.

$$\Pr(T \leq 1500) = \Pr\left(B \leq \frac{1500}{n} - \frac{N}{n^2}\right).$$

Figure 2 displays the probability of not running out of tests as a function of N, for two values of p. If $p = 0.02$ and we set $n = 8$, we can test about 5000 individuals with probability larger than 0.95. If $p = 0.002$ and we set n to 23, we can test about 14600 individuals with 1500 tests with probability larger than 0.95.

The Dorfman approach, although simple, allows for the testing of a large number of individuals with a limited number of tests. For a given infection probability and test constraint, we can determine optimal pool sizes as well as the number of individuals to test. If we are in a scenario with 1500 tests and $p = 0.02$, as one recent paper from India suggests [9], we can test around 5000 individuals using pool sizes of 8, with high probability. Note this is over a three-fold increase in the number of individuals compared to individual testing. Pool sizes between 5 and 32 are in line with recent medical literature on pooled testing for SARS-CoV-2, which we outline in the next section.

Several alternatives and improvements to Dorfman testing exist. Sterrett testing [10] begins similarly to Dorfman by pooling N individuals into N/n pools of size n for an ini-

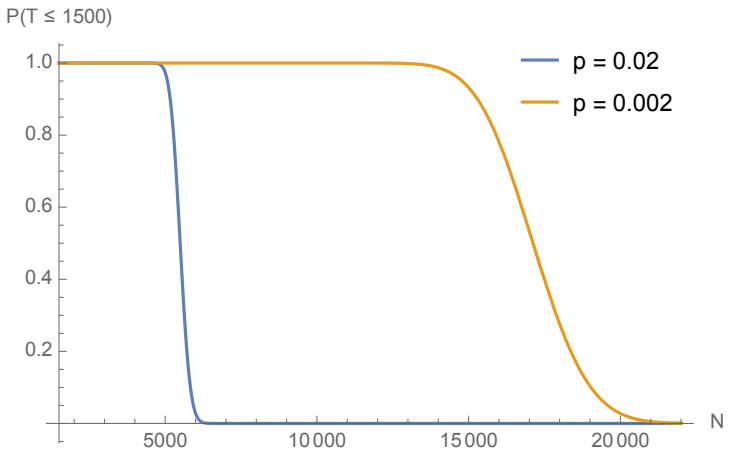

Figure 2: Probability the number of tests needed to test N individuals is less than 1500 as a function of N for varying values of p, the infection probability.

tial round of testing. If a pool is positive, the individuals samples are tested sequentially until a positive sample is found. The remaining individual samples are pooled and tested together. Testing stops if the new pool is negative. If the pool is positive, the procedure is repeated. Halving algorithms [11] provide another alternative to the Dorfman approach. Like in the Dorfman approach, halving algorithms pool samples for initial testing. Individual samples of the initial positive pooled samples are then pooled into two new equal or almost equal sized pools for retesting. If one or both of the new pools is positive, the process is repeated. The alternative methods described in this paragraph provide improvements over Dorfman's procedure but, unlike Dorfman's procedure, they may require more than two tests per individual.

Several methods use heterogeneity in the risk levels of samples to improve the expected number of tests needed. If the probability of infection varies across samples and this information is available or can be estimated, high probability samples should be tested individually while low probability samples should be pooled and Dorfman tested [12]. Even coarse grained separation of samples into high, medium, and low risk can provide benefits. Other approaches combine risk heterogeneity with Sterrett testing [13] and risk heterogeneity with halving methods for additional gains [14]. These procedures provide efficiency gains at the cost of increased complexity of the testing procedure.

It is also possible to use pooled testing to estimate p, the infection probability, without any retesting [15]. In this approach, the fraction of positive pooled tests provides an estimator for q, the probability of at least one infected sample in a group of size n. A consistent estimator

of p can be derived from the estimator of q. A potential limitation of this method is that it does not provide individual test results for positive groups.

Biomedical considerations

Pooled sample testing has already been conducted for SARS-CoV-2 in several countries including the US, Israel, and Germany. In the US, researchers at the Stanford Health Care Clinical Virology Laboratory tested 2888 individual samples from the San Francisco Bay Area using a pooled approach [6]. 2740 nasopharyngeal (throat) and 148 bronchoalveolar lavage (lung) samples collected between January 1, 2020 and February 26, 2020 were pooled in groups of 9 or 10 to create 292 pools. The pools were tested using reverse transcriptase-PCR (RT-PCR). Two of the pools tested positive, requiring the testing of their respective individual samples. Two individual samples were positive for a confirmed positivity rate of 0.07% (2/2888). One pool test resulted in a false positive.

Researchers at Rambam Health Care Campus, Israel, recently demonstrated a single positive SARS-CoV-2 sample can be detected in pool sizes of up to 32 samples using RT-PCR [7]. Nostril and throat swabs were first tested individually to identify positive samples. One positive sample was then combined with 1 negative, 3 negative, 7 negative, 15 negative, 31 negative, and 63 negative samples to create pooled samples of size 2, 4, 8, 16, 32, and 64. Ten replicates of each sample size were then tested. Nine of the ten 32 sample pools correctly tested positive, indicating a false negative rate of 10% and suggesting consistent detection. In addition, seven of the ten 64 sample pools tested positive. However, the researchers caution additional PCR amplification cycles may be needed for pooled samples of size 64. Their manuscript provides a detailed description of their RT-PCR procedure.

Researchers at the German Red Cross Blood Donor Service and the University Hospital Frankfurt at Goethe University have stated infected samples can be detected via RT-PCR in pools of size 5 [8]. However, no manuscript of the work is available at time of writing. According to news reports, Nebraska's Public Health Lab is pooling samples of size 10 for SARS-CoV-2 detection [16].

Conclusion

Pooling sample testing allows for increased testing capacity while preserving limited resources including testing reagents, budget, and time. Dorfman testing is both simple to implement and effective when the infection probability of samples is low. Optimal pool sizes can be

determined along with maximal number of individuals that can be tested. Several improvements of Dorfman testing exist that trade off increased efficiency with increased complexity. Recent medical research has confirmed pooled sample testing using RT-PCR, the testing method recommended by the World Health Organization (WHO) and the Indian Council of Medical Research (ICMR), is viable and effective for SARS-CoV-2 detection.

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