



## Original Article

# The best pooling strategy to reduce polymerase chain reaction tests during the coronavirus disease-19 pandemic at low prevalence

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Submission : 18-Sep-2024  
Revision : 21-Oct-2024  
Acceptance : 12-Nov-2024  
Web Publication : 23-May-2025

## ABSTRACT

**Objectives:** Pooling can reduce reverse transcriptase polymerase chain reaction (RT-PCR) tests during the coronavirus disease-19 (COVID-19) pandemic. Pooling strategy is a complex issue. Recent advances in computer science may provide a better strategy. **Materials and Methods:** We developed our algorithm which can help healthcare workers set up their pooling policy during the COVID-19 pandemic. **Results:** Comparing with three other strategies, naming single pooling, array pooling, and hypercube pooling, our multiple pooling shows to be the best with minimal RT-PCR tests per patient. **Conclusion:** We hope clinicians in COVID-19 pandemic regions can use our algorithm to reduce both RT-PCR tests and time and hence save more lives.

**KEYWORDS:** *Coronavirus disease-19, Efficient pooling polymerase chain reaction, Multiple pooling polymerase chain reaction*

## INTRODUCTION

The world has just gone over the coronavirus disease-19 (COVID-19) pandemic. We are sure that another pandemic will come not far away. In the beginning, when the prevalence is low and the reverse transcriptase polymerase chain reaction (RT-PCR) kits are not enough, it is the best scenario for pooling RT-PCR.

Pooling is a useful way to reduce RT-PCR tests during the COVID-19 pandemic [1-20]. Single pooling strategy is widely used. Most of the government choose pooling sizes 5–10. Israel chose up to 64 pooling sizes [21]. Rwanda chose up to 100 pooling sizes. Array pooling is also well-known. Indeed, array pooling is an extreme form of hypercube pooling with dimension two [1].

Single pooling is widely known and used. We pool samples with certain sample size. Collect positive patients, and then, we test every positive pooled test [6].

Some researchers believe that positive patients could be only one or two per pool. Thus, they suggested another pooling can be done. To make the second pooling sample dividable, they chose the second pooling size as a factor of the first pooling size.

Array pooling is another strategy; we put  $n \times n$  samples on the  $n \times n$  arrays. Then, pool each row and each column for PCR test. By locating both the positive column number and positive row number, we can locate the positive patient.

However, we usually face the difficulties of:


1. How to pool? Array pooling? Two stages pooling? Three stages? Or more?
2. What is the best pool size?

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**How to cite this article:** Chu CL, Wu TY, Hou SM, Shih KS, Chu YL, Chen CK, et al. The best pooling strategy to reduce polymerase chain reaction tests during the coronavirus disease-19 pandemic at low prevalence. Tzu Chi Med J 2025;37(3):299-303.

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	DOI: 10.4103/tcmj.tcmj_244_24

3. How many RT-PCR tests can be saved?

These questions are both biological and mathematical. This study aimed to build a program to answer these questions and to facilitate the application of pooling from the perspective of mathematics. By answering these questions, we propose our invention “Multiple Pooling Strategy.”

**MATERIALS AND METHODS**

Our study contains no human data. Thus, Institutional Review Board approval is not needed.

Before developing new methods, we need to know the evaluation function. For the pooling strategy, the evaluation function is “expected PCR tests for each sample.” If it is larger than one, it means we need to do more than one PCR test for one patient. In this situation, we do not need pooling. The lower the “expected PCR tests for each sample”, the more efficient the pooling strategy is.

Another important issue is “maximal allowed dilution.” Pooling will dilute the sample and thus will increase the cycle threshold value and decrease the sensitivity. However, it will not decrease specificity. Therefore, before we pool the sample, we must test the RT-PCR machine and calculate the sensitivity of dilution. With acceptable sensitivity, we can get the “maximal allowed dilution.” If the pooling number is smaller than it, we do the pooling. If the pooling number is larger, then we should do the pooling with “maximal allowed dilution” as the pooling number.

According to a previous report, we know the mathematics of a single pooling strategy [6]. It shows that pooling is effective only when the prevalence is smaller than 0.3. We need Lambert W function, and the mathematics is complex. On the other hand, we need to know the next step after first pooling. There is no mathematical formula; for this, we can only get it by brute force method with computer. If the conditional probability after the previous pooling is smaller than 0.3, then we need another pooling until it is no smaller than 0.3. The algorithms are shown:

$E(p)$  means the best pooling size with prevalence  $p$ .

$E(p) = 1$  when  $P > 0.3$

$E(p) = n, n = m$  of the minimum element of the set

$$\{(1/m + E(p / (1 - (1 - p)^m))) | m = 2, 3, 4, \dots\}$$

We begin the calculation with  $P = 0.3$ , and then  $P$  decreases gradually. There exists the best solution, thus we steadily increase  $m$ . The element of the set will decrease to a minimum and then begin to increase again. We stop at the time of the turning point.

The flowchart is also shown in Figure 1.

We set up the algorithm using C++ language on the Windows platform and then stored the results for comparison.

Another issue is array pooling. We pool  $n \times n$  array, do the tests for every row and column, and then do the tests individually for samples with positive rows or columns. We also built a program for array pooling.

The expected RT-PCR tests for each sample

$$= (\text{row test} + \text{column test} + \text{positive row column test})/n^2$$

$$= (n + n + [\text{expected positive rows}] [\text{expected positive columns}])/n^2$$

$$= 2/n + (1 - [1 - p]^n)^2 \tag{1}$$

Where  $n$  is array’s row and column number, and  $p$  is the prevalence rate. To find the minimal value, we need to calculate its first-degree differential and assume it to be zero.

$$d(2/n + [1 - (1 - p)^n]^2)/d(n) = 0$$

To solve it, we need the Lambert W function again, which is hard to implement. Thus, we turned to the other way, which is to calculate every situation and choose the best results, the brute force method. With the calculation power of contemporary computers, the algorithm is easy to be implemented.

**RESULTS**

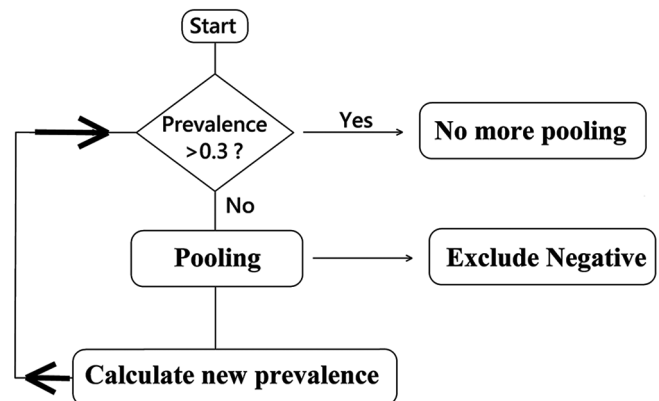
We show the usage of the algorithm in four scenarios.

**Multiple-stage pooling without maximal allowed dilution**

This is used when we only know the prevalence rate. We just input the prevalence rate. If the prevalence rate after the first pooling is still low, we suggest second-stage pooling, as shown in the flowchart [Figure 1]. This goes on until the prevalence rate is high enough and the pooling strategy has no further effect [Table 1]. Multiple pooling strategy is used to know the prevalence rate only with unlimited maximal allowed dilution. In this example, the prevalence rate is

**Table 1: Multiple pooling strategy with prevalence rate of 0.01**

Pooling	Prevalence	Size	Total tests
1	0.01	69	0.014493
2	0.019993483	34	0.029203
3	0.040248674	17	0.043818
4	0.080079554	8	0.059428
5	0.164388478	4	0.074636
6	0.320788261	1	0.105809



**Figure 1:** Flowchart of multiple pooling. When the prevalence is <0.3, we calculated the pooling number from the algorithm and do the pooling. Then we exclude the negative samples, calculate the new prevalence, and then repeat the procedure

0.01 [Figure 2]. The strategy showed first-stage RT-PCR tests by pooling with 69 samples. Then, we excluded negative samples and collected the positive samples for the second stage by pooling with 34 samples. Then, we excluded negative samples and collected the positive samples for the third stage by pooling with 17 samples. Then, we excluded negative samples and collected the positive samples for the fourth stage by pooling with 8 samples. Then, we excluded negative samples and collected the positive samples for the fifth stage by pooling with 4 samples. Then, we did the rest of the positive samples without pooling. In total, we can finish RT-PCR tests with only 0.1058 tests per sample, which means that we can save 89.4% on RT-PCR tests.

**Pooling with maximal allowed dilution**

This is used when we know the prevalence rate and the maximum allowable dilution. This may happen when the calculated pooling size is larger than our machine limit, i.e. the maximum allowable dilution. For example, if our RT-PCR machine could only detect 10 pooling samples and the calculated result was 11, then, we need to adjust the prevalence rate and the pooling size. The result is shown in Table 2. In this example, the prevalence rate was 0.01 and the maximum allowed dilution was 10. The strategy showed first-stage RT-PCR tests by pooling with 10 samples. Then, we excluded negative samples and collected the positive samples for the second stage by pooling with 6 samples. Then, we excluded negative samples and collected the positive samples for the third stage by pooling with 3 samples. Then, we did the rest of the positive samples without pooling. Totally, we could finish RT-PCR tests with only 0.1554 tests per sample. That is, we can save 84.5% RT-PCR tests.

**Array pooling**

With prevalence rate, we can calculate the array size. In this example, the prevalence rate is 0.01 [Figure 2]. The result

**Table 2: Multiple pooling strategy with prevalence rate of 0.01 and maximum allowed dilution 10**

Pooling	Prevalence	Size	Total tests
1	0.01	10	0.1
2	0.104582901	6	0.115936
3	0.215817006	3	0.131382
4	0.416818465	1	0.155373

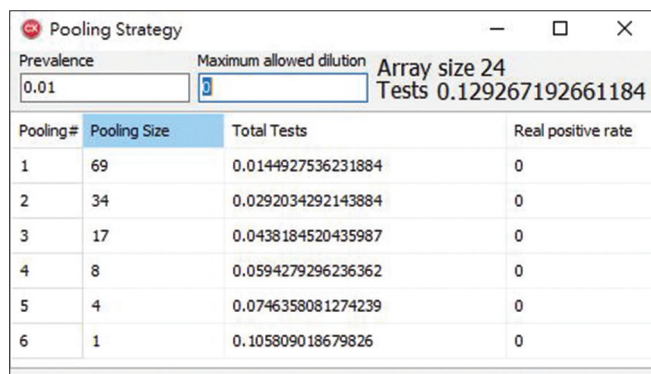


Figure 2: Snapshot of our program

showed first-stage RT-PCR tests by pooling 24 × 24 arrays. Thus, we could do with only 0.129 tests per sample and saved 87.1% RT-PCR tests.

**Array pooling with known array**

If the pooling array number is beyond the RT-PCR machine or operators’ limit which becomes unacceptable, we may need to control the array size. By Equation (1), we could calculate array size and the result showed that we could do with only 0.2335 tests per sample and saved 76.7% RT-PCR tests with prevalence 0.02.

We applied this algorithm from prevalence rate = 0.0001–0.0200 with 0.0001 interval. Figure 3 shows that multiple-level pooling is better than hypercube pooling [12,13,18].

**DISCUSSION**

RT-PCR tests remain the gold standard for diagnosing COVID-19 infection. Countries with rapid growth of infected patients usually face a shortage of RT-PCR test resources, not only the RT-PCR machine but also the time. During the pandemic, delay of diagnosis costs a lot of lives.

We are pretty sure that pandemics will come again and again in the near future. A similar scenario will also repeat again and again. At the beginning of the pandemic, prevalence is low and the testing kit is limited. In this case, pooling the samples for RT-PCR tests is a promising solution. However, most public health authorities are not familiar with how to pool, single or multiple pooling? Array pooling? Pooling number? Column or row number of array pooling? Our study helps the public health authorities in forming their pooling strategies.

Before using our algorithm, the users must know their RT-PCR machines’ limit, which is the maximum allowable dilution. It depends on the required sensitivity. In low prevalence area, the pool size could be larger, sometimes even larger than the machines’ limit. In this situation, we can adjust the pool size.

Another issue is operators’ limit. For the array pooling strategy, the complexity of the samples is row number × column number, which is usually larger than 100.

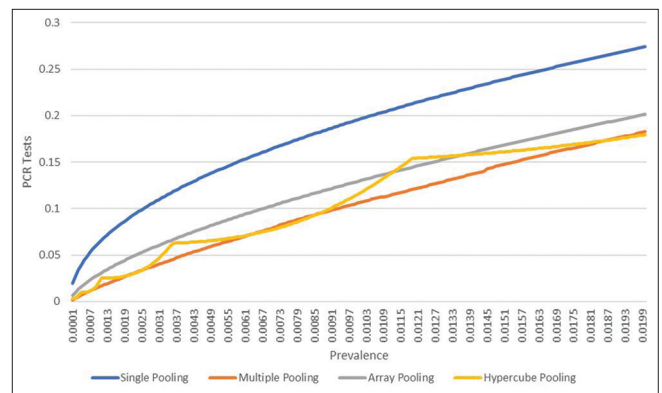


Figure 3: Single pooling, multiple pooling, array pooling, and hypercube pooling. All have good performance. Multiple pooling is the best, hypercube pooling is the second [12,13]

We should keep in mind this issue and avoid any operational mistakes in managing these samples.

There is a website calculator (<https://gillingscovid19.unc.edu/research-app/covid-19-testing-pooling-calculator>). It works well, however, it does not provide multiple-level pooling larger than three. In our experience, it happens when the prevalence rate is smaller than 0.082 and is common. Furthermore, Internet may not be readily available in some areas. In these situations, our algorithm works better.

Previous reports used hypercube pooling [1,12,13,18]. Mathematically, its performance is good [Figure 3]. However, it is really hard for human beings to do hypercube pooling with four, five, or higher dimensional operations.

In this study, multiple-stage pooling is the best strategy with the lowest RT-PCR tests and minimal operational complexity. The operators may just exclude the negative pooling samples in every iteration and then collect the positive samples for the next pooling operation. The procedure is much easier than array pooling and hypercube algorithm. The only disadvantage is multiple stages, which may not be suitable for emergent usage.

To save the RT-PCR kits, the best performance is our invention, multiple pooling. Does it also save time? It depends on the available resources. If we have unlimited RT-PCR machines, we can do all RT-PCR tests for all patients at the same time. Our strategy does not save time in this world with unlimited RT-PCR machines. However, if we have only one RT-PCR machine, our strategy does save time. These are the two extreme situations. Usually, we have some RT-PCR machines, not only one. Therefore, our strategy saves time in the majority of real-world settings.

We implement of the algorithm on Windows platform using C++. We would like to provide it to our readers as freeware [Figure 2]. We would like to send the executable file to readers by e-mail.

The algorithm is effective in every situation and should be easy to implement for other platforms such as iPhone Operation System or Android.

## CONCLUSIONS

Our algorithm shows good performance. We hope it can be used widely, not only for the COVID-19 pandemic but also for other pandemics in the future, when PCR tests are needed for the diagnosis of men while having limited PCR resources.

### Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

### Financial support and sponsorship

This work was funded by grants from the Taiwan Ministry of Science and Technology (grant number 109-2314-B-038-029).

### Conflicts of interest

There are no conflicts of interest.

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