Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Pooled Testing Strategies for SARS-CoV-2 diagnosis: A comprehensive review

Evangeline Ann Daniel, Bennett Henzeler Esakialraj L, Anbalagan S, Kannan Muthuramalingam, Ramesh Karunaianantham, Lucia Precilla Karunakaran, Manohar Nesakumar, Murugesan Selvachithiram, Sathyamurthi Pattabiraman, Sudhakar Natarajan, Srikanth Prasad Tripathy, Luke Elizabeth Hanna*

Department of HIV/AIDS, National Institute for Research in Tuberculosis, Chennai, India

ABSTRACT

SARS-CoV-2 has surged across the globe causing the ongoing COVID-19 pandemic. Systematic testing to facilitate index case isolation and contact tracing is needed for efficient containment of viral spread. The major bottleneck in leveraging testing capacity has been the lack of diagnostic resources. Pooled testing is a potential approach that could reduce cost and usage of test kits. This method involves pooling individual samples and testing them 'en bloc'. Only if the pool tests positive, retesting of individual samples is performed. Upon reviewing recent articles on this strategy employed in various SARS-CoV-2 testing scenarios, we found substantial diversity emphasizing the requirement of a common protocol. In this article, we review various theoretically simulated and clinically validated pooled testing models and propose practical guidelines on applying this strategy for large scale screening. If implemented properly, the proposed approach could contribute to proper utilization of testing resources and flattening of infection curve.

© 2021 Elsevier Inc. All rights reserved.

Keywords:
SARS-CoV-2
COVID-19
Pooled testing
RT-PCR
Diagnosis
Sensitivity

ARTICLE INFO

Article history:
Received 3 March 2021
Accepted 9 May 2021
Available online 17 May 2021

Contents
1. Introduction ............................................................... 2
2. Pooled testing strategies ............................................................... 2
2.1. Adaptive testing ............................................................... 2
2.1.1. Dorfman pooling ............................................................... 2
2.1.2. Binary splitting ............................................................... 2
2.1.3. Multistage testing ............................................................... 2
2.1.4. Household grouping ............................................................... 2
2.1.5. Challenges in adaptive testing ............................................................... 2
2.2. Non-Adaptive Testing ............................................................... 3
2.2.1. Matrix testing ............................................................... 3
2.2.2. Hypercube algorithm ............................................................... 3
2.2.3. Compressed sensing ............................................................... 3
2.2.3.1. P-BEST (Pooling-Based Efficient SARS-CoV-2 Testing) algorithm ............................................................... 3
2.2.3.2. Tapestry ............................................................... 3
3. Validation of Pooled Testing in clinical samples ............................................................... 4
4. Discussion ............................................................... 4
5. Practical guidelines for employing Pooled Testing for COVID-19 diagnosis ............................................................... 6
6. Conclusion ............................................................... 7

Authors' Contribution ............................................................... 7
Funding ............................................................... 7
Supplementary materials ............................................................... 7
References ............................................................... 7

* Corresponding author. Tel.: +91-98-0644-376; fax: +91-44-28362528.
E-mail addresses: evangelineanndaniel@gmail.com (E.A. Daniel), hannatrc@yahoo.com (L.E. Hanna).

https://doi.org/10.1016/j.diagmicrobio.2021.115432
0732-8893/© 2021 Elsevier Inc. All rights reserved.
1. Introduction

A novel coronavirus was recognized as the pathogen responsible for the chronic cases of pneumonia that were reported in Wuhan, China in late December 2019 (Andersen et al., 2020; Zhou et al., 2020). This virus was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Lü et al., 2020) causing coronavirus disease 2019 (COVID-19). Due to its extensive transmission across the globe, COVID-19 was declared as a pandemic by the World Health Organization on 11 March 2020 (Cucinotta and Vanelli, 2020). As of 9 February 2021, the WHO has reported 105.4 million cases and 2.3 million deaths worldwide (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports). Owing to limited monitoring capacity, diagnostic testing is often limited to symptomatic individuals or people with underlying risk factors for chronic diseases and healthcare workers. A significant proportion of potentially infectious carriers in the community remain undiagnosed, since a majority of population are asymptomatic or present with mild symptoms (Wu and McGoogan, 2020).

However, it has been documented that asymptomatic individuals can very well transmit SARS-CoV-2 (Rothe et al., 2020; Ye et al., 2020). With gradual evolution of the pandemic over the last few months, diagnostic centres encounter new challenges in performing surveillance screening of asymptomatic population, driving test numbers even beyond those executed during the peak of the pandemic. Therefore, it is vital to amplify the screening capacity for index case isolation and contact tracing to prevent further spread. The primary diagnostic test that has been employed worldwide to screen COVID-19 is the Real-Time Reverse Transcriptase–Polymerase Chain reaction (RT–PCR) with acceptable sensitivity and specificity levels (Corman et al., 2020). But even in developed countries, testing by RT-PCR is highly constrained due to shortage of reagents and the limited tests that can be performed in a single run. In addition to this, low-income countries with inadequate resources cannot afford the test. Therefore, a rapid, widespread and cost-effective testing strategy is required to curb further spread. A promising strategy to escalate screening capacity is to pool multiple samples in a single test.

Pooled testing (pooling or group testing) involves the screening of a sample pool comprising of multiple individual samples, followed by individual testing (pool deconvolution) only of pools that test positive. A pool that tests negative implies a negative result for all individual samples in the pool. This potentially leads to a much larger number of people being tested when compared to individual testing, while keeping the number of tests the same thereby accelerating the throughput of the existing testing infrastructure (Hanel and Thurner, 2020). Pooling bears the advantage of estimating the positivity rate in a population with fewer tests. It also reduces the testing time. This strategy of grouping samples was pioneered by Robert Dorfman in 1943 to screen men with syphilis in the US military (Dorfman, 1943). Henceforth, pooling of samples has been used as a screening strategy in multiple infectious diseases like HIV (Emmanuel et al., 1988; Litvak et al., 1994; Sherlock et al., 2007; Stramer et al., 2004), Influenza (Hourfar et al., 2007; Van et al., 2012), Malaria (Taylor et al., 2010) and various bacterial diseases (Currie et al., 2004; Edouard et al., 2015; Singer et al., 2006).

In this review article, we present multiple pooled testing approaches reported so far for the diagnosis of COVID-19. We evaluate the efficiency of these methods and provide insights on their practical relevance. We also provide a meta-analysis of various clinical studies that successfully employed pooled testing. Using the practical guidelines furnished, pooling protocols can be framed by testing centres to increase their diagnostic capacity.

2. Pooled testing strategies

Effective application of pooled testing requires baseline parameters like the prevalence rate of the population, sensitivity and specificity of the test and limit of detection. A key step in this approach is to ascertain an ideal pool size that conserves maximum resources with consistent testing performance (Abdalhamid et al., 2020b). In multiple recent articles and preprints, ideal batch sizes for different ranges of prevalence along with the expected reduction in fraction of tests and costs are put forth based on various algorithms (Aragón-Caqueo et al., 2020; Brynildsrud, 2020; Gu et al., 2020; Rai et al., 2020; Regen et al., 2020; Tan et al., 2020).

Pooled testing works on two paradigms, adaptive and non-adaptive testing. Adaptive pooling is a sequential multi-stage grouping design. In this method, the pool results of the former stage impact the pooling strategy of the consecutive stages. Non-adaptive pooling methods involve parallel testing of multiple pools in a single stage.

2.1. Adaptive testing

In this approach, the tests are performed sequentially. A group is randomly selected based on predicted prevalence rate and tested. The outcome of this test determines the next group to test and so on. Thus, the size and samples of a pool are chosen adaptively based on a previous pool and its test outcome (Mentus et al., 2020).

2.1.1. Dorfman pooling

Dorfman Pooling is the simplest form of pooled testing wherein a set of individual samples are clustered into a pool. If the pool tests positive, it is deconvoluted to decrypt the positive individuals. If the pool is tested negative, all the individuals in the group are interpreted as negative (Dorfman, 1943). Dorfman testing is the most preferred pooling method in clinical scenarios although it usually utilises the highest number of tests among all pooling approaches.

2.1.2. Binary splitting

In Binary splitting, instead of performing individual tests of a positive group as in Dorfman’s method, the pool is further divided into two equal sized sub-pools and tested again. The sub-pool that tests positive is split in the next stage until all the negative pools are excluded (Litvak et al., 1994). Binary method is feasible with a maximum of four steps since several dividing steps can be time consuming.

2.1.3. Multistage testing

Multistage model involves sampling different pool sizes at different stages contrary to the binary split method, which uses just two pools in every stage. The number of tests and stages are also substantially minimized. A three-stage testing scheme with pool size of 16 samples in a prevalence rate of 5% has an efficiency gain of 3. A lower prevalence rate favours large pool size and multiple stages while smaller pool sizes with lesser number of stages are to be chosen in higher prevalence settings (Eberhardt et al., n.d.). Gajpal et al employed dynamic programming and have furnished details on the optimum stages required for different sample sizes (Gajpal et al., 2020).

2.1.4. Household grouping

In case of a homogenous population like a locality or neighbourhood, an exposed individual has high chances of spreading the disease immediately to families, neighbours, etc. In this approach, the first step involves forming optimum sized pools of a homogenous population followed by swab tests for all members of the pool. In a second step, positively tested pools will be retested individually. In a population of 150 000, this method can lead to reduction of existing tests by around 56% in moderate prevalence settings with pool sizes up to 25 (Deckert et al., 2020; Takyi-Williams, 2020).

2.1.5. Challenges in adaptive testing

The storing of multiple samples and rerunning of RT-PCR makes lab organization for adaptive approach a resource intensive process.
The diagnosis process is liable to delays because of the follow-up loop. Fig. 1A-C represents various adaptive pooled testing models that are implemented for COVID-19 diagnosis.

2.2. Non-Adaptive Testing

In the non-adaptive approach, each sample is distributed over multiple pools, and all the tests are simultaneously run in parallel yielding reliable results after one round of testing. With increase in pool size and sample multiplicity, a gradual rise in the number of false positives is expected. But initial observation of the prevalence rate and calculation of the explicit error bounds of the false positives leads to streamlined testing and reduced detection times for mass screening (Taüfer, 2020).

2.2.1. Matrix testing

Matrix or array testing is a high throughput non-adaptive screening procedure originally proposed by Phatarfod and Sudbury (Phatarfod and Sudbury, 1994). This approach involves constructing a matrix-like grid of samples and grouping samples within rows and columns of the matrix. Unlike adaptive testing, where individuals are consigned to one pool, here each sample will be distributed in two or more individual pools. Samples that traverse the positive rows and columns are tested individually to diagnose the positive cases whereas samples present outside the row-column intersections are declared negative.

2.2.2. Hypercube algorithm

A recent article proposes to arrange samples in the geometry of a hypercube by grouping along its hyperplanes thereby allocating each member in pool sizes of three or more. Based on the viral prevalence, a pool size $N = 3^D$ ($D$= dimension) is determined initially. With decrease in prevalence, $D$ rises, leading to reduced number of tests. Fig. 2 depicts pooling based on hypercube algorithm. This algorithm was validated in oropharyngeal swabs in Rwanda, with precise diagnostic accuracy even after a 100-fold dilution (adding 99 negative samples to one positive sample). In a prevalence setting of less than 0.01%, an improvement of 400 times efficiency compared to regular testing was observed (Mutesa et al., 2021). But an intersection of any two hyperplanes in more than one point might occur in dimensions above three. This might impair testing efficiency by creating unnecessary correlations between different pools.

2.2.3. Compressed sensing

In compressed sensing, the result is not just a binary reading (positive or negative), but also reveals the viral loads (copies/mL) of individuals who test positive (Ghosh et al., 2020a; Yi et al., 2020).

2.2.3.1. P-BEST (Pooling-Based Efficient SARS-CoV-2 Testing) algorithm. In this approach, samples are stratified on the basis of Reed-Solomon error correction. P-BEST was tested using four sets of 384 samples, each containing positive cases ranging from two to five. This method was able to correctly identify all positive cases using only 48 tests with a testing efficiency gain of 8. Modelling algorithms report very less false positive and negative numbers (<2.75 and <0.33 respectively) utilising P-BEST decoding (Shental et al., 2020).

2.2.3.2. Tapestry. Tapestry pooling is a single round testing where each individual is assigned to three different groups. The algorithm takes ct values from the pooled RT-PCR tests as input and yields the output result. If the sample is tested positive, an estimated viral titre is also provided. An app has also been developed to facilitate...
implementation in clinical laboratory settings. The method has been shown to work in vitro with zero false positives and false negatives. Validation in clinical samples is ongoing (Ghosh et al., 2020b; Gopalkrishnan and Krishna, 2020).

3. Validation of Pooled Testing in clinical samples

A foremost requisite of any modelling algorithm is the successful translation of theoretical simulations into clinically relevant applications. There are effective feasibility studies of various pooling methods in SARS-CoV-2 diagnosis. Studies have also compared pooled testing with naive testing to ensure that detection accuracy is not compromised. Table 1 summarizes different studies worldwide that have validated pooled testing in various pool sizes and prevalence rates. Four different pooling strategies have been employed so far in clinical setups (Fig. 3). The most common method is to collect the samples and pool them just before extracting RNA. Few studies have reported pooling the extracted RNA and then proceeding for RT-PCR. Gupta et al have reported 95.4% sensitivity in detecting positive samples while pooling RNA keeping individual testing as reference (Gupta et al., 2020). Though the turn-around time is swift in this method, it does not save many resources. Swab pooling in a single tube at the time of collection is another practice. This is found to be as sensitive as individual testing (Christoff et al., 2021) but if a positive pool is encountered, samples have to be collected again. Hence double swabs have to be collected from every individual in the beginning (Chen et al., 2020). A couple of studies have directly collected the samples in lysis buffer instead of the viral transport media (VTM) to avoid dilution. This direct pooling also inactivates the virus easing the extraction process (Schmidt et al., 2020; Wacharapluesadee et al., 2020). Owing to the non-invasive nature of sample collection, the feasibility of utilising pooled saliva has also been tested and found that detection is possible without much compromise in sensitivity (Barat et al., 2020; Fogarty et al., 2020; Pasomsub et al., 2020). Studies have reported improved test turn-around time and conservation of laboratory resources while adapting pooled testing (Abdalhamid et al., 2020a; Agoti et al., 2020). The diagnostic accuracy while adapting pooled testing is found to be highly efficient in most of the studies (Fig. 4). The WHO has furnished an acceptable level of ≥80% sensitivity and ≥97% specificity for COVID-19 diagnostics in the Target Product Profile (TPP).

Other works over the last few months have suggested refined models, from a more theoretical viewpoint with real world data as example. In a 2% prevalence rate, a matrix-based 96 well pooling is predicted to reduce tests up to four-fold while an eight-fold reduction is expected at 0.5% prevalence (Sinnott-Armstrong et al., 2020). A simulation study based on the available positivity data proposes an optimum pool size of up to 32 for a population of 10 000 (Bukhari et al., 2020). With the underlying prevalence rate of 2% in South Korea, sample stratification in pools up to 16 using a multi-stage scheme allows a five-fold efficiency gain relative to individual testing (Eberhardt et al., n.d.). Other simulation models and algorithms have also been proposed (Chow and Chow, 2021; Nalbantoglu, 2020; Pilcher et al., 2005; Polage et al., 2020; Vukičević and Polašek, 2020).

4. Discussion

The diagnostic screening of SARS-CoV-2 can be performed in a lesser time span by pooling samples. It is also evident that testing in pools substantially reduces the number of reagents, labour costs and resources. However, pooled testing is efficient only in low prevalence settings with least number of positive pools. Larger number of positive pools might lead to exhaustive deconvolution for retesting individual samples. Though several novel algorithms have been simulated as a strategy for pooled testing, Dorfman’s Pooling method with certain improvements remain the gold standard for pooled testing. A major challenge with pooled testing is the increase in false negative rates since grouping multiple negative specimens with few positives could lead to undetectable levels of the viral load. But studies investigating the accuracy of pooled testing in wide ranges of dilutions have revealed false-negative rates below 10%, suggesting that the dilution introduces minimal error (Bateman et al., 2020; Smalley et al., 2021; Yelin et al., 2020). The general documented challenges during individual testing include low viral load in some patients, errors during RT-PCR testing, and RNA degradation due to improper handling. These effects might be augmented in pooled testing procedures, so it is even more crucial to carefully monitor the testing process. Further inevitable practical difficulties are time constraints, sample conservations for multi-stage pooling, tracking and retesting individuals of positive pools. Many studies have reported models based on the assumption of the presence of one positive sample per pool. It is important to tailor algorithms for more than one positive case. These are some points to ponder to
Table 1
Summary of global pooled testing studies - Validation of pooled testing in different clinical settings. Pooling works with maximum efficiency in prevalence <3% with pool size <10

| Study setting | Location | Sample size | Sample type | Prevalence (%) | Optimum Pool size | Reduction in tests (%) |
|---------------|----------|-------------|-------------|----------------|-------------------|------------------------|
| Pooling samples before RNA extraction | | | | | | |
| (Abdalhamid et al., 2020) | Nebraska, US | 60 | Nasopharyngeal swabs | 11 | 80 | |
| (Abid et al., 2020) | Tunisia, North Africa | 800 | Nasopharyngeal swabs | <1 | 10 | 80 |
| (Agoti et al., 2020) | Kilifi, Kenya | 1500 | Nasopharyngeal swabs | <4 | 6 | 53.3 |
| (Barak et al., 2020) | Jerusalem, Israel | 133816 | Nasopharyngeal swabs | 1-6 | 5.8 | 76 |
| (Lohse et al., 2020) | Homburg, Germany | 1191 | Nasopharyngeal and Oro-pharyngeal swabs | 1.93 | 30 | 78 |
| (Hogan et al., 2020) | San Francisco Bay Area, California, US | 2888 | Nasopharyngeal and bronchial lavage swabs | 0.07 | 9, 10 | 77 |
| (Ben-Ami et al., 2020) | Jerusalem, Israel | 26576 | Nasopharyngeal swabs | 0.23 | 8 | 85 |
| (de Salazar et al., 2020) | Spain | 3519 | Nasopharyngeal swabs | 6.86 | 10 | 61.57 |
| (Barat et al., 2020) | Maryland, Washington D.C., US | 449 | Saliva | 5 | 5 | 40 |
| (Ho et al., 2021) | Hong Kong, China | 55 | Deep throat saliva | 0.39 | 5 | - |
| (Borillo et al., 2020) | California, US | 101 | Nasopharyngeal, mid-turbinate swabs | ≤10 | 4 | - |
| (Chen et al., 2020) | Wuhan, China | 23 | Oropharyngeal swabs | - | 6, 10 | - |
| (Chikatara et al., 2021) | Chandigarh, India | 500 | Nasopharyngeal swabs | ≤2 | 10 | 69 |
| (Chong et al., 2020) | Maryland, US | 7000 | Nasal mid-turbinate and nasopharyngeal swabs | 0.11 | 10 | 89 |
| (Chong et al., 2020) | Maryland, Washington D.C., US | 449 | Saliva | 5 | 5 | 40 |
| (Lim et al., 2020) | Malaysia | 1745 | Nasopharyngeal and oro-pharyngeal swabs | ≤5 | 10 | 57 |
| (Mastrianni et al., 2020) | New York, US | 530 | Nasopharyngeal and oro-pharyngeal swabs | 1-2 | 3 | 67 |
| (Mohanty et al., 2020) | Odisha, India | 7228 | Nasopharyngeal and oro-pharyngeal swabs | 3.5 | 4 | 64.2 |
| (More et al., 2021) | Oklahoma, US | 630 | Nasopharyngeal swabs | ≤8 | 5 | 52.5 |
| (Mutesa et al., 2021) | Rwanda, East Africa | 1280 | Oropharyngeal swabs | 2 | 20 | - |
| (Perchetti et al., 2020) | Seattle, US | 160 | Nasopharyngeal swabs | -8 | 4 | - |
| (Singh et al., 2020) | Madhya Pradesh, India | 1000 | Nasopharyngeal and oro-pharyngeal swabs | 4.8 | 5 | 70 |
| (Shental et al., 2020) | Beer-Sheva, Israel | 1115 | Nasopharyngeal and oro-pharyngeal swabs | 1 | 48 | 87.1 |
| (Thanh et al., 2021) | Ho Chi Minh, Vietnam | 96123 | Nasopharyngeal swabs | 0.1 | 5 | 77 |
| (Torres et al., 2020) | Valencia, Spain | 40 | Nasopharyngeal swabs | 10 | 5, 10 | - |
| (Volpato et al., 2020) | Porto Alegre, Brazil | 220 | Nasopharyngeal and oro-pharyngeal swabs | 0 | 10 | - |
| (Wang et al., 2020) | New Haven, US | 1648 | Nasopharyngeal and oro-pharyngeal swabs | 19.1 | 4, 8 | - |
| (Watkins et al., 2020) | Da Nang, Vietnam | 10265 | Nasopharyngeal swabs | 10 | 5 | 47 |
| (Yelin et al., 2020) | Haifa, Israel | 388 | Nasopharyngeal and oro-pharyngeal swabs | - | 32 | - |
| (Denny et al., 2020) | Duke University, Durham, North Carolina | 10265 | Nasopharyngeal swabs | ≤0.1 | 5 | 90 |
| (Schneitler et al., 2020) | Karlsruhe, Germany | 25978 | Nasopharyngeal and oro-pharyngeal swabs | 0.9 | 10 | 91 |

Pooling extracted RNA

| Location | Sample size | Sample type | Prevalence (%) | Optimum Pool size | Reduction in tests (%) |
|----------|-------------|-------------|----------------|-------------------|------------------------|
| Bangkok, Thailand | 200 | saliva | 9 | 5 | 47.5 |
| Ultrakhand, India | 102 | Nasopharyngeal swabs | 4.1 | 20 | 40 |
| Chile, South America | 63 | Nasopharyngeal swabs | 10 | 5 | - |
| New Delhi, India | 280 | Nasopharyngeal and oro-pharyngeal swabs | - | 8 | 85 |
| Freiburg, Bonn, Leipzig, Germany | 700 | Pharyngeal swabs, sputum, broncho-alveolar lavage fluid | - | 10 | 99.89 |
| Spain | 100 | Nasopharyngeal swabs | 2 | 20 | 77 |
| New Delhi, India | 60 | Nasopharyngeal and oro-pharyngeal swabs | 1.7 | Sub Pool 5 | 80 |
| Galapagos, Equador | 114 | Nasopharyngeal swabs | ≤5 | 3 | - |

(continued)
implement pooled testing strategy over the ongoing individual testing method.

5. Practical guidelines for employing Pooled Testing for COVID-19 diagnosis

With gradual relaxation in lockdown rules, reopening of Universities and schools and resumption of transport, it is critical to ramp up the numbers of testing of asymptomatic population. By comparing various pooled testing strategies implemented so far, we put forth the optimal choice for various scenarios.

Select Pooling method

For community surveillance in resource limited settings with inadequate testing facilities, a door-to-door approach is suitable. Asymptomatic people in households and localities can be tested using this approach. Individual samples from houses in close vicinity can be pooled together considering the spatial clustering of the outbreak in a particular area. This can be organized by mapping the region, plotting the households in the locale and pooling samples from adjoining houses.

Binary splitting algorithms can be employed to test market vendors, bank and supermarket agents and other frontline workers who were actively exposed during the lockdown enforced by the Government.

Routine screening of health care workers and hospital staff can be done with Matrix Pooling since it involves a single round of tests.

When the testing speed is a primary factor, it may be feasible to employ Multistage testing as it enables us to perform parallel group tests

Table 1 (Continued)

| Study setting | Location                  | Sample size | Sample type                          | Prevalence (%) | Optimum Pool size | Reduction in tests (%) |
|---------------|---------------------------|-------------|--------------------------------------|----------------|-------------------|------------------------|
| (Schmidt et al., 2020) | Frankfurt, Germany       | 100         | Nasopharyngeal and oropharyngeal swabs | 2              | 5, 10             | 40                     |
| (Wacharapluesadee et al., 2020) | Bangkok, Thailand       | 99          | Nasopharyngeal and oropharyngeal swabs | 0.1-10         | 10                | 80                     |

* indicates that studies that did not report prevalence rate at the time of testing and the resources saved

Fig. 3. Pooling methods employed in clinical setups: (A) Sample pooling before RNA extraction (B) Pooling of extracted RNA (C) Sample pooling at the time of collection (D) Sample collected directly into the lysis buffer; VTM= viral transport media.
Hypercube algorithm can find application in regular testing of players and staff of sports teams.

*Estimate the Positivity rate*

At the beginning of each test, it is vital to estimate the positivity rate. Based on the empirical results received from a specific laboratory in a population, the rate of positive tests should be updated on a daily basis.

*Define the maximal Pool size*

Medical councils from across the globe have recommended to use a pool size of 5. But in areas with prevalence lesser than 1%, the pool size can go up to 10 based on clinical validation studies around the world. It is always important to restrict pooled testing approach for prevalence up to 5%. As prevalence increases, the efficiency of the pooled testing flattens.

Web based applications like shiny app (https://bilder.shinyapps.io/PooledTesting/) (Hou et al., 2020) can also be used to determine the efficient pool size based on parameters like prevalence, sensitivity and specificity of the assay.

*Proceed with RT-PCR of each pool, neglect negative pools, deconvolute positive pools.*

6. Conclusion

Testing in pools has the potential to substantially expand the testing capacity and lower the limitations present in the current diagnostic tests for SARS-CoV-2. Studies have reported considerable increase in testing throughput and detection efficiency with consistent levels of sensitivity and specificity. Thus, pooled testing is a feasible method to circumvent the bottlenecks in the existing testing methods of COVID-19. Given the successful implementation of this strategy in different countries, the approach may find applicability in routine community surveys.

**Authors’ Contribution**

Evangeline Ann Daniel: Conceptualization, Design and Methodology Validation, Data collection, curation and Analysis, Writing Original Draft

Bennett Henzeler Esakialraj L: Conceptualization, Design and Methodology Validation, Data collection, curation and Analysis, Writing Original Draft, Visualization

Anbalagan S: Data collection, curation and Analysis

Kannan Muthuramalingam: Data collection, curation and Analysis

Ramesh Karunaianantham: Data collection, curation and Analysis

Lucia Precilla Karunakaran: Data collection, curation and Analysis; Reviewing and editing draft

Manohar Nesakumar: Data collection, curation and Analysis; Reviewing and editing draft

Murugesan Selvachithiram: Data curation and analysis

Sathyamurthi Pattabiraman: Data curation and analysis

Sudhakar Natarajan: Data curation and analysis; Reviewing and editing draft

Luke Elizabeth Hanna: Conceptualization; Supervision; Reviewing and editing draft

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2021.115432.

**References**

Abdalhamid B, Bilder CR, Garrett JL, Iwen PC. Cost effectiveness of sample pooling to test for SARS-CoV-2. J Infect Dev Ctries 2020a;14:1136–7. doi: 10.3855/jidc.13935.

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 2020b;153:715–8. doi: 10.1093/ajcp/aqaa064.

Abid S, Ferjani S, El Moussi A, Ferjani A, Nasr M, Landolsi I, et al. Assessment of sample pooling for SARS-CoV-2 molecular testing for screening of asymptomatic persons...
Denny TN, Andrews L, Bonsignori M, Cavanaugh K, Datto MB, Deckard A, et al. Implementation of a pooled surveillance testing program for asymptomatic SARS-CoV-2 infections on a college campus - duke university, Durham, North Carolina, August 2-10, 2020. MMWR Mortal Morb Wkly Rep 2020;69:1743–7. doi: 10.15585/mmwr.mm6946e1.

Dorfman R. The detection of defective members of large populations. Ann Math Stat 1943;14:36–40. doi: 10.1214/aoms/1177731136.

Eberhardt BN, Breuckmann NP, Eberhardt CS. Multi-stage group testing improves efficiency of large-scale COVID-19 screening. J Clin Virol n.d. https://doi.org/10.1016/j.jcv.2020.104382.

Edouard S, Prudent E, Gautret P, Memish ZA, Raoult D. Cost-effective pooling of DNA from nasopharyngeal swab samples for large-scale detection of bacteria by real-time PCR. J Clin Microbiol 2015;53:1002–4. doi: 10.1128/JCM.03609-14.

Eis-Hüübing AM, Honemann M, Wenzel J, Berger A, Widera M, Schmidt B, et al. Ad hoc laboratory-based surveillance of SARS-CoV-2 by real-time RT-PCR using mini-pools of RNA prepared from routine respiratory samples. J Clin Virol 2020;127: 104381. doi: 10.1016/j.jcv.2020.104381.

Emmanuel [C], 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;41:582–4.

Farfan MJ, Torres JP, Ojha A, Mavrides M, Gallardo P, Laster J, et al. Optimizing RT-PCR detection of SARS-CoV-2 for developing countries using pool testing. Rev Chilena Infectol 2020;37:276–80. doi:10.1016/j.rchipl.2020.03.007.26.

Fogarty A, Joseph A, Shaw D. Pooled saliva samples for COVID-19 surveillance programme. Lancet Respir Med 2020;8:1078–80. doi:10.1016/s2213-2600(20)30444-4.

Freire-Pasquell B, Vega-Mariño P, Velez A, Cruz M, Garcia-Bereguain MA. Sample pooling of saliva RNA extracts to speed up SARS-CoV-2 diagnosis using CDC FDA EUA RT-PCR kit. Virus Res 2020;290: 198173. doi:10.1016/j.virusres.2019.18173.

Gajpal Y, Appadoo SS, Shi V, Liao Y. Optimal multi-stage group partition for efficient coronavirus screening. Norwich, NY: Social Sci Res Network 2020. doi: 10.2139/ssrn.390691.

Garg A, Ghoshal U, Patel SS, Singh DV, Arora AK, Vasanth S, et al. Evaluation of seven commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens. J Med Virol 2020; doi:10.1002/jmv.26691.

Ghosh S, Agrawal R, Rehan MA, Pathak S, Agrawal P, Gupta Y, et al. A compressed sensing approach for group testing of COVID-19 detection. ArXiv 2020:057895 [q-Bio].

Ghosh S, Rajwade A, Sirkar Krishna, Gopalkrishnan N, Schaus TE, Chakravarty A, et al. Tapestry: A single-round smart pooling technique for COVID-19 testing. MedRxiv 2020. doi:10.1101/2020.05.16.20099655.

Gopalkrishnan M, Krishna S. Pooling Samples to Increase SARS-CoV-2 Testing. J Indian Inst Sci. 2020;100:787–92. doi:10.14745/717-009-2020-020024.

Graham M, Williams E, Isles N, Baudromé E, Tohtu T, Druce J, et al. Sample pooling on the Cepheid Xpert Xpress SARS-CoV-2 assay. Diagn Microbiol Infect Dis 2021;99:115238. doi:10.1016/j.diagmicrobio.2020.115238.

Griesemer SB, Syke CV, George KS. Assessment of sample pooling for clinical SARS-CoV-2 testing. J Clin Microbiol 2021. doi:10.1128/JCM.02612-20.

Gu T, Yao L, Zhang Y, Fracc G, Thomson D, Li J, et al. A cost-effective plan for global COVID-19 testing - an infection rate stratified, algorithm guided, multiple-level, continuously pooled testing strategy. Sci Total Environ 2020;765:144251. doi:10.1016/j.scitotenv.2020.144251.

Guo R, Padhi A, Khodare A, Agrawal R, Ramachandran K, Mehta V, et al. Pooled RNA reverse transcriptase quantitative real-time PCR assay for SARS-CoV-2 infection: A reliable, faster and economical method. PLOS ONE 2020;15:e0238417. doi:10.1371/journal.pone.0238417.

Gupta E, Padhi A, Khodare A, Agrawal R, Pathak S, Agrawal P, Gupta Y, et al. A compressed-sensing approach to group-testing for COVID-19 detection. ArXiv 2020:057895 [q-Bio].

Gupta S, Farfan MJ, Torres JP, Ojha A, Mavrides M, Gallardo P, Laster J, et al. Optimizing RT-PCR detection of SARS-CoV-2 for developing countries using pool testing. Rev Chilena Infectol 2020;37:276–80. doi:10.1016/j.rchipl.2020.03.007.26.

Gupta S, Agrawal R, Rehan MA, Pathak S, Agrawal P, Gupta Y, et al. A compressed-sensing approach for group-testing of COVID-19 detection. ArXiv 2020:057895 [q-Bio].

Gur A, Goyal R, Goyal D, Singh S, Khosla P, Amarnath P, et al. Comparison of three commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens. J Med Virol 2020; doi:10.1002/jmv.26691.

Ghos G, Agarwal R, Rehan MA, Pathak S, Agrawal P, Gupta Y, et al. A compressed-sensing approach for group-testing of COVID-19 detection. ArXiv 2020:057895 [q-Bio].

Hahn R, Thurner S. Boosting test-efficiency by pooled testing for SARS-CoV-2. – Formula for optimal pool size. PLOS One 2020;15:. doi:10.1371/journal.pone.0238417.

Hara GA, Saboo MK, Pinsky BA. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA 2020. doi:10.1001/jama.2020.5425.

Hirotsu Y, Maehima M, Shibusawa M, Nagakubo Y, Hosaka K, Amemaya K, et al. Pooling RT-qPCR testing for SARS-CoV-2 in 1000 individuals of healthy and infection-suspected patients. Sci Rep 2020;10:18899. doi:10.1038/s41598-020-76043-2.

Ho YL, Wong AH, Tang KFS, Wong RCW, Leung ECM, Lai RWM. Comparison of three commercial SARS-CoV-2 assays for pooled testing of deep throat saliva for surveillance of patients attending general outpatient clinics. J Med Virol 2021. doi:10.1002/jmv.26764.

Hogan GA, Saboo MK, Pinsky BA. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA 2020. doi:10.1001/jama.2020.5425.

Hou P, Tebbbs JM, Wang D, McManus CS, Bilder CR. Array testing for multiple assays. Bioinformatics 2020;26:417–31. doi:10.1093/bioinformatics/btt665.

Houfek MK, Theman A, Ekelund M, Puthavathana P, Laue T, Seifried E, et al. Blood Screening Influenza. Emerg Infect Dis 2007;13:1081–3. doi: 10.3201/eid1307.060851.

Khodare A, Padhi A, Gupta E, Agrawal R, Dubey S, Sarak SK. Optimal size of sample pooling for SARS-CoV-2 RNA testing: An avant-garde for scaling up severe acute respiratory syndrome coronavirus-2 testing. Indian J Med Microbiol 2020;38:18–23. doi: 10.1016/j.ijmmmb.2020.03.007.26.

Kim SY, Lee J, Sung H, Lee H, Yang M, Goo YK, et al. Pooling upper respiratory specimens for rapid mass screening of SARS-CoV-2 by real-time RT-PCR. Emerg Infect Dis 2020;26:2469–72. doi:10.3201/eid2609.200195.

Li H, Sun K, Persing DH, Tang Y-W, Shen D. Real-time Large-scale Screening of Specimen Pools for Coronavirus Disease 2019 (COVID-19) Infection on Sanya Airport, Hainan Island. China. J Clin Virol 2020;130:104380. doi:10.1016/j.jcv.2020.104380.

Lim KL, Johari NA, Wong ST, Khaw LT, Tan BK, Chan KK, et al. A novel strategy for community screening of SARS-CoV-2 (COVID-19): Sample pooling method. PLOS ONE 2020;15:e0238417. doi:10.1371/journal.pone.0238417.
