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Review

Pooled Testing Strategies for SARS-CoV-2 diagnosis: A comprehensive review

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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.

Contents

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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) (Lu 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). Thenceforth, 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 (Täufer, 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 = 3D (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

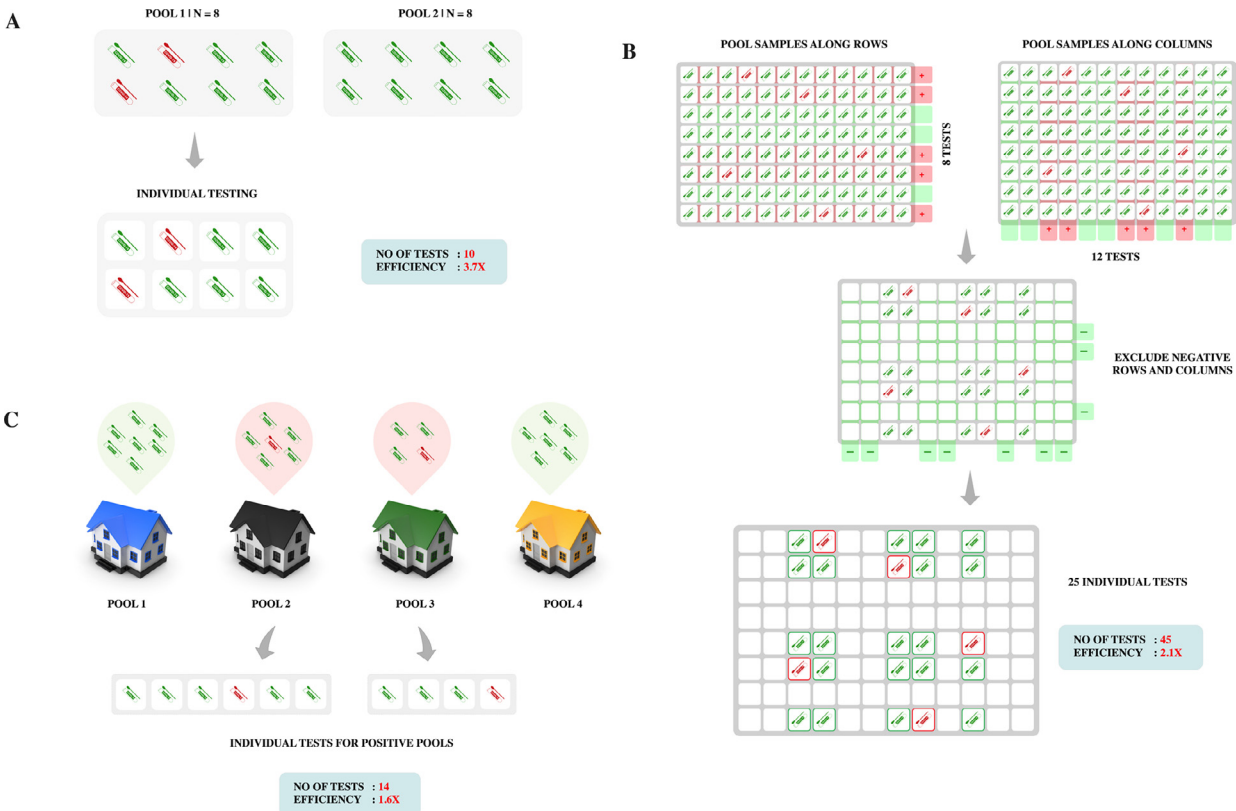

Fig. 1. Adaptive pooled testing strategies: (A) Dorfman Pooling: Population is divided into two pools of optimum size 8 and tested. The positively tested pool is then deconvoluted to identify the individual infected sample. (B) Column and row pooling: On a 96-well plate, rows and columns are pooled separately. The negative rows and columns are excluded. The individuals from positive rows and columns are then tested individually. In the above illustration, 5 positive samples are identified with 45 tests, compared to 96 tests if tested individually. (C) Household grouping: Pools are formed from each household in a locality and tested. Positive groups undergo individual testing.

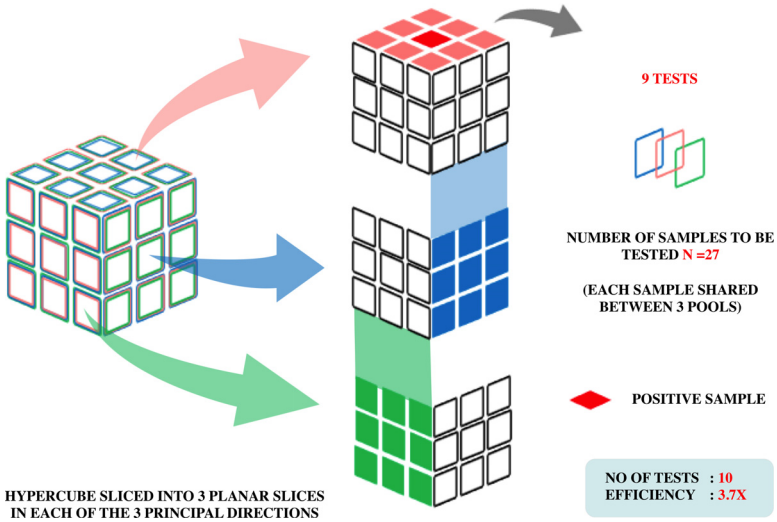

Fig. 2. Adaptive pooled testing strategy: Sample pooling based on Hypercube algorithm. For this example, the three sets of slices are shown in blue, red and green. If one infected individual is present, tests on each set of slices identify their coordinate in that direction. Hence only nine tests would uniquely identify them. (Colour version of figure is available online)

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 naïve 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 turnaround 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 rate 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., 2020b)	Nebraska, US	60	Nasopharyngeal swabs	1	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 bron-choalveolar 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, midturbi-nate swabs	≤10	4	-
(Chen et al., 2020)	Wuhan, China	23	Oropharyngeal swabs	-	6,10	-
(Chhikara et al., 2021)	Chandigarh, India	500	Nasopharyngeal swabs	<2	10	69
(Chong et al., 2020)	Melbourne, Australia	29000	Nasopharyngeal swabs	<0.5	8	87.5
(Cleary et al., 2020)	Massachusetts, US	960	Nasopharyngeal swabs	3.1	48	65
(Das et al., 2020)	Maryland, US	7000	Nasal mid-turbinate and nasopharyngeal swabs	0.11	10	89
(Graham et al., 2021)	Melbourne, Australia	31	Nasopharyngeal swabs	<5	4,6	>50
(Hirotsu et al., 2020)	Tokyo, Japan	555	Nasopharyngeal swabs	3.6	5,10	47
(Kim et al., 2020)	Seoul, Jeonju, South Korea	350	Nasopharyngeal and oro-pharyngeal swabs	-	2-16	-
(Li et al., 2020)	Hainan, China	944	Nasopharyngeal swabs	Airport	10	87.6
(Lim et al., 2020)	Malaysia	1745	Nasopharyngeal and oro-pharyngeal swabs	<5	10	57
(Mastrianni et al., 2020)	New York, US	530	Nasopharyngeal 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 10	52.5 45
(Mutesa et al., 2021)	Rwanda, East Africa	1280	Oropharyngeal swabs	2	20	-
(Perchetti et al., 2020)	Seattle, US	160	Nasopharyngeal swabs	<8	4	-
(Praharaj et al., 2020)	India	1000	Nasopharyngeal and oro-pharyngeal swabs	-	5 10	-
(Procop et al., 2021)	Ohio, US	20	Nasopharyngeal swabs	0.5	10	
(Salimnia et al., 2021)	Detroit, US	15	Nasopharyngeal swabs	2	6	70
(Singh et al., 2020)	Madhya Pradesh, India	545	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)	Da Nang, Vietnam	96123	Nasopharyngeal swabs	<1	5	77
(Torres et al., 2020)	Valencia, Spain	40	Nasopharyngeal swabs	-	5, 10	-
(Volpato et al., 2020)	Porto Alegre, Brazil	220	Nasopharyngeal and oro-pharyngeal swabs	-	10	-
(Wang et al., 2020)	San Francisco Bay, California, US	1648	Nasopharyngeal and oro-pharyngeal swabs	19.1	4, 8	47
(Watkins et al., 2020)	New Haven, US		Saliva	>3 <1	5, 10, 20	-
(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)	Homburg, Germany	25978	Nasopharyngeal and oro-pharyngeal swabs	0.9	10	91
Pooling extracted RNA						
(Pasomsub et al., 2020)	Bangkok, Thailand	200	Saliva	9	5	47.5
(Deka et al., 2020)	Uttarakhand, India	102	-	4.1	20	40
(Farfan et al., 2020)	Chile, South America	63	Nasopharyngeal swabs	10	5	-
(Gupta et al., 2020)	New Delhi, India	280	Nasopharyngeal and oro-pharyngeal swabs	-	8	85
(Eis-Hübinger et al., 2020)	Freiburg, Bonn, Leipzig, Regensburg, Frankfurt (Germany)	700	Pharyngeal swabs, sputum, broncho-alveolar lavage fluid	-	10	99.89
(Cabrera Alvargonzalez et al., 2020)	Spain	100 60	Nasopharyngeal swabs	2 1.7	20 Sub Pool 5	77 80
(Khodare et al., 2020)	New Delhi, India	55	Nasopharyngeal and oro-pharyngeal swabs	4	6	63
(Freire-Paspuel et al., 2020)	Galapagos, Equador	114	Nasopharyngeal swabs	<5	3	-

(*continued*)

Table 1 (*Continued*)

Study setting	Location	Sample size	Sample type	Prevalence (%)	Optimum Pool size	Reduction in tests (%)
Pooling at the time of collection in a single VTM tube						
(Christoff et al., 2021)	Brazil	19535	Nasopharyngeal swabs	1.26	16	77
(Alcoba-Florez et al., 2020)	Tenerif, Spain	4475	Nasopharyngeal swabs	5	5	62
(Garg et al., 2020)	Lucknow, India	19570	Nasopharyngeal and Oro-pharyngeal swabs	<5	5 10	76 93
(Griesemer et al., 2021)	New York, US	20	Upper respiratory swab	<1.5	5, 9	75
(Agarwal et al., 2021)	Delhi, India	230	Nasopharyngeal swabs	-	5	70
Pooling directly into lysis buffer						
(Schmidt et al., 2020)	Frankfort, Germany	100	Nasopharyngeal and oro-pharyngeal swabs	2	5, 10	40
(Wacharapluesadee et al., 2020)	Bangkok, Thailand	99	Nasopharyngeal and oro-pharyngeal swabs	0.1-10	10	80

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

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

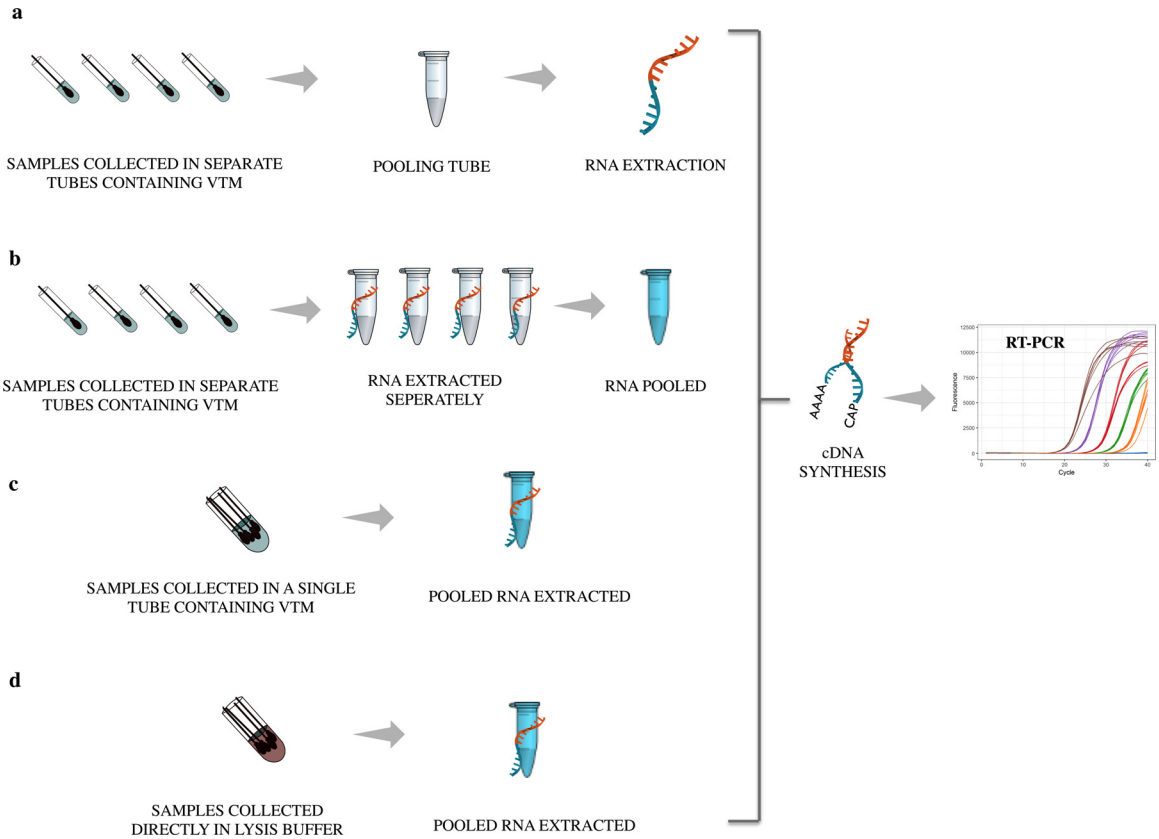

Fig. 3. Pooling methods employed in clinical setups: (A) Sample pooling before RNA extraction (B) Pooling of extracted RNA (C) Sample pooling at the time of collection (D) Sample collected directly into the lysis buffer; VTM= viral transport media.

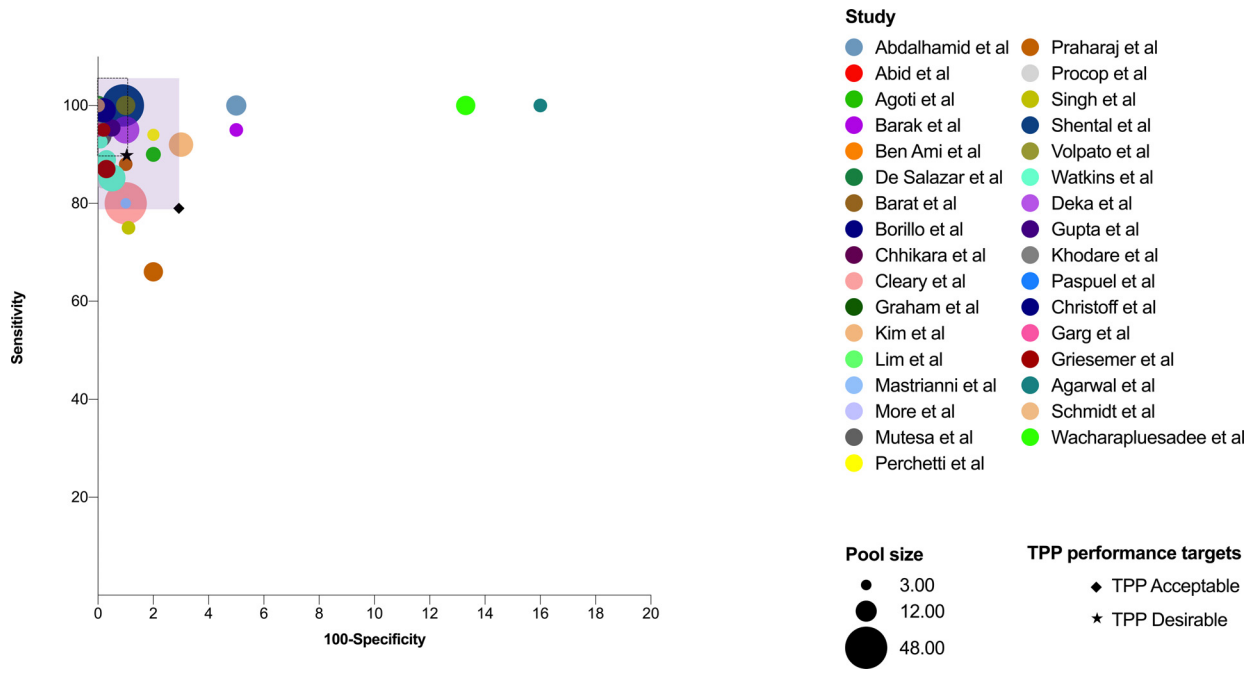

Fig. 4. Diagnostic accuracy utilising pooled testing strategy: The size of the bubble depicts pool size, while the different colours code for different studies. The purple-shaded area represents areas with sensitivity and specificity combinations that meet the acceptable levels of Target Product Profile (TPP) put forth by WHO for COVID-19 diagnostics. The dashed square box within the purple-shaded area portrays the desirable level. Study bubbles clustered within the acceptable limits infer efficient diagnostic capability in detecting SARS-CoV-2 by pooling samples. (Colour version of figure is available online)

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

Srikanth Prasad Tripathy: Supervision; Reviewing and editing draft

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

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Supplementary materials

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