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Strategies for Scaling up SARS-CoV-2 Molecular Testing Capacity

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KEYWORDS

• Automation • Capacity • Molecular testing • Pooling • RT-PCR, SARS-CoV-2

KEY POINTS

- Pooling of specimens successfully expanded SARS-CoV-2 testing capacity during a time of supply shortages.
- Rapid implementation of automation provided additional capacity for testing.
- Sensitivity and specificity were maintained with both pooling and automation.

INTRODUCTION

More than 413 million people have been infected with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) resulting in more than 5.8 million deaths worldwide.[1] Testing for the virus at high volumes has been essential in the battle against the pandemic, yet there continues to be huge variation in the ability of different countries to keep up with their testing needs. The number of tests per confirmed case has varied widely as has the total cumulative cases per country. In March 2020, the United States was averaging 21 tests per confirmed case whereas Taiwan was performing 211 tests per confirmed case. Nearly 1 year later at the beginning of February 2021, the United States was still performing just 12 tests per confirmed case compared with Australia, with 451 tests per confirmed case. Importantly, the overall volume of testing in the United States was quite large, with more than 457 million SARS-CoV-2 tests performed by mid-June 2021.[2] Target populations for testing were highly diverse and included patients being admitted to a hospital or being evaluated in a clinic, asymptomatic individuals at work, or students in educational institutions. In this review, we will address the approaches used to increase the capacity of molecular testing for viral RNA, as this remains an important and challenging problem and the lessons learned in the response to SARS-CoV-2 are applicable to future infectious disease pandemics.

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Strategies used to scale up testing capacity

The following strategies were used to increase the volume of SARS-CoV-2 testing:

- Pooling
- Diversification of platforms
- Decentralization away from public health laboratories
- Conversion of research laboratories into clinical laboratories
- Maximizing number of samples per plate when supplies were low by adjusting the plate layout
- Production of viral transport media in house
- Use of phosphate-buffered saline instead of viral transport media
- Production of 3D-printed swabs
- Validation of assays with lower number of targets
- Multiplexing and automation
- Applying innovative technologies to COVID-19 diagnostics, such as clustered regularly interspaced short palindromic repeats (CRISPR)-based platforms

We will discuss each of these in turn with a focus on pooling.

POOLING

Pooled testing has been used for many years and is ideally suited for situations in which the prevalence of positive samples for an infectious disease is low enough to result in an overall savings of reagents. Models for optimal pool sizes date back nearly 80 years, and testing of blood donations for HIV and hepatitis are excellent examples of successful pooling strategies.[3] Pooling reduces the expense of testing and conserves scarce reagents, which has been critical during the SARS-CoV-2 pandemic when supplies were limiting the testing capacity in many locations. The advantages of robust pooling can be quite dramatic, reducing the number of tests by 90% in low-risk groups.[4]

The following parameters should be considered when adopting a pooling strategy:

- Pooling method (original specimens vs extracted RNA)
- Pooling algorithm
- Size of pool
- Sensitivity of the pooled test

Pooling Method

Pooling is a testing method that combines specimens from multiple subjects into a pool for a single test. When a pool tests negative, the testing is complete for all individual samples in the pool. If the pool tests positive, further testing is required to identify which specimens led to the pool turning positive. An alternative approach is to perform nucleic acid extraction on all specimens individually and then combine the purified products for amplification and signal detection. Studies evaluating the performance of pooled testing have largely focused on nasopharyngeal or midturbinate specimens, but saliva has also been evaluated and shown to pool successfully.

Several groups have developed modifications to traditional algorithms in their efforts to optimize the efficiency of pooled testing strategies. Volpato and colleagues[5] examined pools of 10 nasopharyngeal swabs and found slightly better sensitivity when pooling specimens before extraction compared with testing pooled RNA after extraction. Sanghani and colleagues[6] proposed using large molecular-weight cutoff centrifugal concentrators to improve sensitivity of pooled samples, but this strategy

did not show an increase in sensitivity and the proposed method, which adds a step in the procedure, was not successfully implemented for a laboratory performing high-capacity testing. Conversely, Sawicki and colleagues[7] concentrated pools of 6 or 9 samples using a centrifugal filter before RNA extraction and reported the ability to detect samples with cycle threshold (Ct) values as high as 34.

Another strategy is to pool at the time of collection instead of in the laboratory. A study by Christoff and colleagues reported testing of more than 18,000 individuals by collecting 2 swabs per person, wherein 1 swab was placed in a pooled tube of 16 swabs and the other swab was inserted into a separate tube for individual testing in case the pool turns positive. Although this approach relieves the burden on the laboratory for pooling, it uses twice as many swabs, which were in very short supply at times during the pandemic, and required that tracking of the 16 samples be done by the collection site. The prevalence of SARS-CoV-2 in this study was ~1%, and this group was able to show this approach increased their capacity 4.4-fold, which includes the reflex testing after pool deconvolution.[8] Most studies used pooling of original specimen before extraction and this approach will be discussed further when considering optimal pool size.

Pooling Algorithm

Quite a few publications have described mathematical models for predicting optimal pool size for the SARS-CoV-2 pandemic. One-dimensional pooling, called the Dorfman approach, is the simplest and most commonly used approach. For this strategy, each positive pool must be deconvoluted by retesting each sample included in the pool to identify the infected individuals. In most cases, each pool contains 5 to 10 samples. As an example, Ben-Ami and colleagues[9]. reported a 7.3-fold increase in throughput by pooling groups of 8 specimens to test over 26,000 samples.

Modifications to the Dorfman algorithm include sequential pooling, which is a two-dimensional multistep approach wherein a positive pool is broken into smaller pools for repeat testing. The downside is that each round of testing increases the turnaround time. Another form of two-dimensional pooling is a geometric scheme, also called matrix or tapestry pooling, that offers a theoretic benefit of additional saved time and supplies (**Fig. 1**).[10–12] Matrix pooling uses combinatorial mathematical theory to put each sample in multiple pools, with no 2 samples together in more than 1 pool. This approach permits the identification of the positive samples in the first round, without deconvolution and retesting,[13] but the complexity of this scheme would make manual pooling by a technologist very challenging. Two-dimensional pooling strategies may be feasible with the aid of robotic pipetting instruments, but there are no published reports of successful implementation of this approach to date. Some models have illustrated the advantages of pooling homogeneous groups in a context-sensitive manner, such as staff working in the same office, for maximal efficiency. Although the theoretic benefits are clear, this method would greatly increase the complexity of the preanalytical steps for many laboratory operations and potentially outweigh the benefits in reagent savings.[14] One commentary supported an algorithm of split pooling over the Dorfman algorithm, suggesting that every pool should be tested twice if negative before reporting the negative result; however, this process would lead to unacceptable delays in result reporting. Furthermore, the investigators claim that modern automated laboratory equipment makes it is easy to carry out split pool testing and does not fully capture the complex realities of sample tracking and workflows in the clinical laboratory.[15]

Others have proposed an approach to optimize the testing strategy by considering prevalence and potentially having a different algorithm for a low-risk versus a high-risk

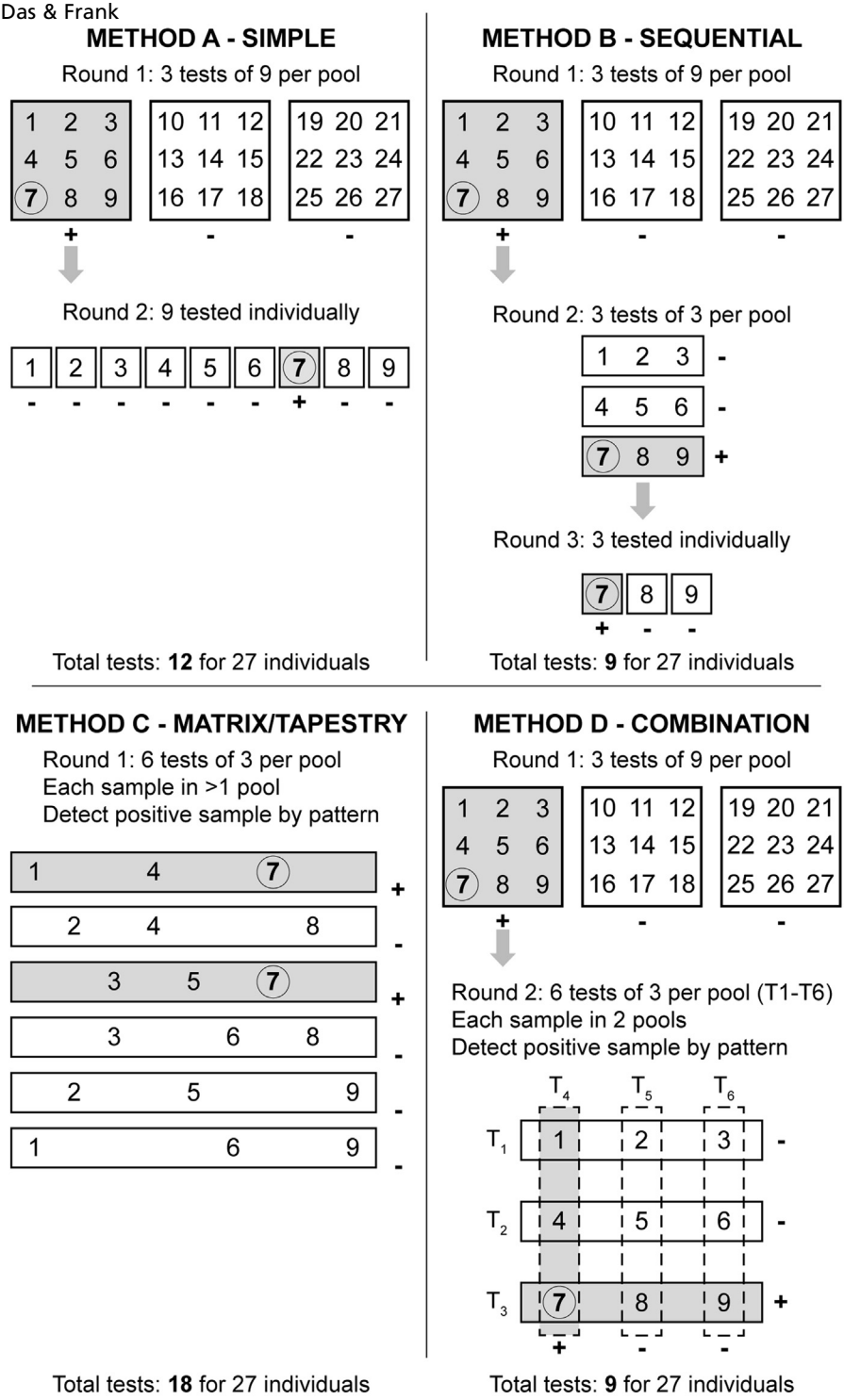

Fig. 1. Algorithms for pooled testing. The simple method is also called the Dorfman method (A). These show simple examples of only one positive (Sample #7) out of 27 samples tested. Methods C and D, in particular, would be more complex as prevalence increases and pools contain multiple positive samples.

population.[4] However, the rapid changing of procedures on a week-to-week basis must consider the training of technical staff and their ability to adjust to modifications in a procedure without error. Automated programmable pooling on an instrument, when available, would reduce the potential element of human error at that step; however, the challenge of rapidly switching between different preanalytical and postanalytical workflows would remain and likely explains why there are very few examples of rapid shifting of pooling algorithms beyond a simple adjustment of pool size with prevalence. Another proposal has been to group samples for pooling by age, but the advantages were shown to be minuscule relative to the extra burden this approach would place on a laboratory.[16] A table comparing efficiency of the Dorman pooling algorithm versus matrix pooling showed variation with prevalence, with the Dorfman method being favored at a low prevalence and the matrix slightly favored at 10% prevalence.[9] Although modeling remains a very valuable tool for exploring many different pooling strategies, empiric studies that have actually validated and implemented pooling are more valuable than simulations.

Size of Pool

The optimal size of a pool depends on the prevalence of the disease as well as the pooling algorithm. As the number of positive pools increases with rising prevalence, the number of tests performed approaches or exceeds that of standard nonpooling methods, thereby negating the savings that would have been realized through more efficient use of reagents. Thus, although the theoretic benefits of pooling have been demonstrated up to a positivity rate of 30%, at such high levels the pool size would need to be exceedingly small to prevent every pool from testing positive and requiring deconvolution. Pool sizes of 10 are optimal over a broader range of prevalence and most studies recommend pooling only if the prevalence is less than 10%.[17,18] Commonly suggested pool sizes range from 4 to 10, though some studies advocate for 32 to 64 samples per pool (**Table 1**).[7,19–27]

The largest real-world study evaluating the performance of a pooled testing strategy comes from Barak and colleagues, who analyzed 17,945 pools created from 133,816 samples drawn from symptomatic and asymptomatic individuals affiliated with the Hadassah Medical Center in Jerusalem, Israel. The investigators used the Dorfman algorithm with pool sizes of either 5 or 8, depending on the prior week's pool positivity rate. Their use of a dynamic pool size, as well as the nonrandom clustering of positive samples based on the location of testing (nursing homes, colleges, and health care settings), resulted in a 76% reduction in the number of polymerase chain reactions (PCR), which exceeded the predicted performance of their strategy. The investigators note the ability to adjust pool size was facilitated by their use of automated liquid handers.[28] A study by Petrovan and colleagues[29] reported efficient detection with pools of up to 80 specimens, but the study validated their protocol using only specimens with high viral loads; therefore, conclusions cannot be generalized to settings in which a significant proportion of specimens are expected to have lower viral loads.

Sensitivity of Pooled Testing

A primary concern with combining multiple specimens into a pool is that it will dilute the signal of individual tests, resulting in the missed detection of low viral load specimens. However, nucleic acid amplification tests for viral RNA are highly sensitive, with a limit of detection as low as 5 copies of virus per reaction. This degree of sensitivity is the reason why pooling has been immensely successful for HIV and hepatitis as there is only a minor loss of sensitivity. With SARS-CoV-2, the realization that specimens with low viral loads are often (though not always) associated with a lower transmission

Table 1
Examples of reports of implemented pooled testing of SARS-CoV-2

Type of Pooling	Pool Size	Specimen Type	Assay	Number Tested	Results	Reference
Simple	5, 10, 15, 5 for large volume analysis	NP	Pathofinder Real Accurate Quadruplex Corona-plus PCR Kit	4475 in 895 pools	Ct ↓ by 2.2, Acceptable for Ct 16.7–39.4	Alcoba-Florez *et al.*[19] 2021
Simple	4	NP MT Nasal	Quest Diagnostics SARS-CoV-2 RNA Qualitative Real-Time RT-PCR	3091	Ct ↓ 1.9/2.38, PPA: 100%	Borillo *et al.*[20] 2020
Simple	5, 7, 10	NP MT	CDC Assay, Panther Fusion SARS-CoV-2	270, then 7000	Ct ↓ by 2.7–3.6 (10 in pool), 0.2–1.8 (5, 7 in pool), Detected all positives with Ct < 36 for all pool sizes	Das *et al.*[21] 2020
Simple	6	NP Saliva	Roche Cobas SARS-CoV-2	564	Sensitivity: NP 100%, Saliva 90%; 25% of samples had Ct > 30	McMillen *et al.*[22] 2021
Simple	5, 10	NP	TaqPath Covid-19 Multiplex Diagnostic Solution	630	Detected Ct 33 consistently for pool of 5, Detected Ct 31 consistently for pool of 10	More *et al.*[23] 2021
Simple	5, 10	Saliva	Sansure SARS-CoV-2 Nucleic Acid Diagnostic Kit	200	Pools of 5 or 10 acceptable	Pasomsub *et al.*[24] 2021
Simple	2, 4, 8, 16, 32, 64	Nasal and Throat	AgPath ID One-Step RT-PCR, WHO primer/probe, BioRad CFX96	72	10% False negative rate for pool of 32, Sensitivity for pool of 16: 96%	Yelin *et al.*[25] 2020

Simple	6, 9	NP, Nasal + OP	Concentrate pool with Amicon Ultra 0.5 mL Ultracell 30K Filter, QIAamp Minicolumn, Z-Path-COVID-19-CE Genesig Real-Time RT-PCR (Primerdesign)	112	Ct decrease 0.5–3, Detected as high as Ct 34	Sawicki *et al.*[7] 2021
Simple	5, 9	Upper respiratory swab	CDC RT-PCR	20 positives into 60 for pools of 5 and into 39 for pools of 9	For CT $\geq$ 33, sensitivity 95% for pools of 5% and 87% for pools of 9	Griesemer *et al.*[36] 2021
Simple	5, 10, 20	Saliva	Luna Universal Probe One-Step RTqPCR, Laboratory Developed primer/probe, Biorad CFX 96 q PCR	23 pools of 5, 23 pools of 10, 31 pools of 20	Sensitivity: 93% for pools of 5%, 89% for pools of 10%, 85% for pools of 20	Watkins *et al.*[33] 2021
Simple	4, 8	NP	Laboratory-developed assay	320	Sensitivity: 75% for pools of 4, 62.5% for pools of 8	Mahmoud *et al.*[37] 2021
Simple	5, 8	NP	Laboratory-developed assay QIAsymphony extraction TaqPath Master Mix QuantStudio 5 LiHa Robot	133,816	Adjusted pool size with prevalence of 0.5%–6%. Spared 76% pf reagents	Barak *et al.*[28] 2021

Abbreviations: positive percent agreement, PPA; nasopharyngeal, NP; mid-turbinate, MT; oropharyngeal, OP; cycle threshold, Ct.

risk paved the way for pooling methods to be accepted by laboratory, hospital, and public health leadership.

Multiple studies have now demonstrated that the slight loss of sensitivity from pooling samplings does not affect the detection of virus from individual samples when they contain RNA levels that correspond to transmissible disease. In most studies this equates to Ct values below 35.[30–32] Importantly, a key decision for an institution is setting the upper bound for Ct value that must be detected in their assay. For example, do specimens with viral loads corresponding to a Ct value of 38 need to be detected, or should only specimens with a Ct value of 34 and lower be considered essential for identification? Ultimately, determining an acceptable loss of sensitivity is a subjective determination of the highest Ct value present in the individuals most likely to spread disease and must be informed by careful epidemiologic studies that are specific to the set of SARS-CoV-2 variants in current circulation.[33]

The key parameter influencing sensitivity is pool size. Wang and colleagues examined a lab-developed test and Panther assays with pools of four reporting a sensitivity of 83%–100% and with pools of eight reporting a sensitivity of 72% to 83%. All false negatives had a Ct value greater than 34.[34] Abdalhamid and colleagues[35] reported that pools of five specimens dropped the highest detectable Ct value of an individual specimen by 0 to 5 cycles although it also reduced the number of tests performed by 69%. Griesemer and colleagues[36] showed pooling of five specimens detected 95% of individual positive samples, but pools of nine detected only 87%. This group focused on identifying samples with Ct values of 33 to 36. Watkins and colleagues[33] reported a sensitivities of 93%, 89%, and 85% for saliva pool sizes of 5, 10, and 20, respectively. A study by Mahmoud and colleagues[37] reported high false negative rates with pools of 4 and 8, but this result was not typical, as the investigators noted difficulty identifying positive samples with a Ct value > 30 whereas many other studies show good sensitivity detecting samples with Ct values as high as 34.

Regulation

From the beginning of the pandemic, the United States Food and Drug Administration (FDA) regulated testing for SARS-CoV-2 for both commercial and laboratory-developed tests because of the potential public health consequences of poor-quality tests used on a massive scale. Early FDA guidance limited pool size to 4 to 8 specimens, even though models suggested that larger pool sizes when deployed in low-prevalence settings would remain effective.[38,39] The recommendations have been since relaxed, but the validation studies require extensive documentation on expected changes in sensitivity, handling of PCR inhibitors, and deconvolution methods.

Preanalytical/Postanalytical Considerations

When properly calibrated to prevalence, pooled testing strategies result in significant savings. However, this comes at the cost of increased preanalytical and postanalytical complexity. Specimen handling becomes more challenging as the pools must be made by an instrument or a technologist. Uncapping and recapping tubes can become a limiting factor for some automated workflows. Furthermore, the larger the pool size, the higher the risk of a specimen mix-up at the time of deconvolution.

A limitation of pooling is that it removes the ability to assess for individual sampling adequacy through a positive internal control, such as the human RNAse P gene. However, our experience has been that the rate of inadequate samples was extremely low for nasopharyngeal, midturbinate, and saliva collections; therefore, this is not likely to be a major drawback. It should be noted that pooling does not reduce the workload for

reporting and billing and instead has increased the work for the information technology staff who must create new data management algorithms for result reporting.[40]

Summary of Pooling

Many laboratories have successfully implemented robust pooling algorithms to efficiently scale up SARS-CoV-2 molecular testing. Lessons learned from the current pandemic will serve to inform laboratories that may need rapid scale-up of testing in the future.

NEED FOR SCALE-UP OF MOLECULAR ASSAYS FOR SARS-CoV-2

Highly sensitive reverse transcriptase-PCR (RT-PCR) testing and contact tracing has been the cornerstone of containment strategies during the pandemic but is predicated on the ability to return accurate results as fast as possible. Mathematical modeling showed that testing delays of more than 3 days significantly reduces the prevention of transmission by contact tracing.[41] Recognizing the need for expanded capacity of rapid testing, initiatives such as the NIH RADx were developed early in the pandemic to speed development of new tests.[42] These and other studies recognized several challenges that would need to be overcome before large scale testing could be instituted.

Challenges to implementation of RT-PCR–based large-scale testing include the availability of high-throughput assays and platforms, adequate access to sufficient reagents, laboratory infrastructure, and the ability for laboratories to develop and validate new assays, the availability of trained personnel and the costs of implementing high capacity population-based testing strategies.[43–45] We consider several strategies to overcome these challenges in the following sections.

STRATEGIES TO CONSIDER FOR LARGE-SCALE TESTING FOR SARS-CoV-2

Increase in Testing Capacity by Modification of Traditional Laboratory-Developed Tests

One of the simplest means of increasing testing capacity is to increase the use of automated RNA extraction methods. Indeed, global shortages and bottlenecks in production of extraction reagents prompted an assortment of studies that investigated alternate extraction procedures or direct PCR amplification on specimens.[20,46] Several studies have evaluated the use of liquid handling robots, describing methods to increase efficiency while reducing dependency on commercial kits. Lazaro-Perona and colleagues evaluated an in-house developed liquid handling system (OT-2) and compared its performance with that of the MagMAX (ThermoFisher Scientific) commercial kit-based extraction platform. The Ct values for the *orf1ab* and S gene targets from clinical specimens were comparable between the 2 methods. The robot required intensive programming that was shared on an open access repository.[47] Borillo and colleagues evaluated a Tecan Evo 150 automated liquid handler (Tecan Group Limited, Männedorf, Switzerland) using the PHASIFY viral RNA extraction kit (PHASE Scientific International Ltd., Hong Kong). This method was found to be superior to extraction using the NucliSENS easyMAG (bioMérieux, Marcy-l'Étoile, France), especially for saliva specimens.[20]

Another simple and effective method to scale up testing would be to remove the extraction procedure altogether by performing RT-PCR directly on the specimen or on minimally processed specimens. Although this strategy does not actually automate the procedure or increase throughput, it does reduce hands-on labor and time while bypassing the reagent supply chain shortages. The key objectives of an extraction

procedure, inactivation of virus and release of RNA, can be achieved by a simple heat inactivation step in the presence of proteinase K as shown by Vogels and colleagues[48] in their SalivaDirect method. Their nucleic acid extraction-free method was successful in detecting SARS-CoV-2 RNA using a dualplex RT-PCR assay with 6 to 12 RNA copies/μL using reagents from multiple vendors. Additional advantages of SalivaDirect were the low supply cost and the stability of the specimen for up to 7 days without compromise in sensitivity. Claas and colleagues[49] evaluated the combination of an automated liquid handling robot, the Tecan Fluent 480 (Tecan Switzerland), with a simplified commercial liquid sample preparation for direct RT-PCR and showed acceptable sensitivity and specificity in SARS-CoV-2 samples with Ct values of less than 33. Use of detergents and guanidinium isothiocyanate with chloroform for direct sample preparation showed variable sensitivity. Of note, some of these studies demonstrated a loss of sensitivity when detergents or other extraction reagents or heat inactivation methods were used in direct sample preparation in RT-PCR.[50–52] Caution and proper biosafety precautions should also be used when using these non-extraction sample preparations to determine the level of viral inactivation.[49,53]

Combining methods to reduce extraction steps and multiplexing several probes in a single reaction is also an effective way of increasing capacity. An additional advantage of multiplexed assays is panel testing that includes other respiratory viral targets. This would significantly increase testing efficiency during the respiratory viral season when influenza or respiratory syncytial virus may be circulating and patients may have similar symptoms early in the disease. Several laboratories validated a multiplexed version of the available SARS-CoV-2 assays before implementation of the assay in clinical care during the initial phases of the pandemic to reduce consumption of resources.[54,55] A newer version of the CDC SARS-CoV-2 assay that received FDA authorization for emergency use in January 2021 is a multiplexed assay for detection of influenza A and B along with SARS-CoV-2 across a broad range of instruments that permit high-throughput extraction. Shortly thereafter, multiplexed commercial assays for respiratory pathogens such as the BioFire Respiratory 2.1 Panel and Xpert Xpress CoV-2/Flu/RSV (Cepheid, Sunnyvale, CA) received FDA authorization and provide rapid actionable results during respiratory season.

Increase in Testing Capacity Using Automated Platforms

As the capacity required to meet demand increased to millions of tests per day, it became clear that the need for reagents and labor would far exceeded the available global supply chain. Automated platforms are capable of significantly increasing throughput while reducing human error and achieving high diagnostic precision. Such platforms have been meaningful during past outbreaks such as Ebola, Zika, and HIV.[42] From these past experiences, we have learned that an ideal diagnostic platform is low complexity, high throughput, random access, able to detect multiple targets in a single run, have limited need for human labor, and occupy a small floor area. There are several FDA-approved high-throughput automated platforms that offer large-scale testing for SARS-CoV-2[56], however, one that satisfies all of the above conditions, while also being affordable and devoid of supply chain issues, does not exist. Most of the existing platforms combine nucleic acid extraction, amplification, detection, analysis, and reporting of results, thus increasing throughput, accuracy, and precision while reducing sources of human error at both analytical and postanalytical steps. The performance characteristics are comparable as shown in recent studies (**Table 2**), but there are differences in the functionality of these platforms, including throughput per 8-h work shift, technician hands-on time, and random-access capability. Many clinical laboratories use multiple platforms simultaneously to efficiently

Table 2
Example of investigations of clinical performance of fully automated platforms for the detection of SARS-CoV-2

Platform(s) Evaluated	Study Design	Type and Number of Specimens	Comparator Method	Results of Study	Reference
Hologic Panther Fusion SARS-CoV-2 Assay (Fusion) Hologic Aptima SARS-CoV-2 Assay (Aptima) BioFire Defense COVID-19 test (Biofire)	Retrospective and prospective	Nasopharyngeal swab (n = 150)	Consensus results from 3 platforms	94.7%–98.7% PPA, 100% NPA	Smith *et al.*[83] 2020
Hologic Panther Fusion SARS-CoV-2 Assay (Fusion) Simplexa COVID-19 Direct (Diasorin) assay GenMark ePlex SARS-CoV-2 (GenMark) assay	Retrospective and prospective	Nasopharyngeal swab (n = 104)	CDC SARS-CoV-2 assay	96%–100% PPA and NPA	Zhen *et al.*[84] 2020
Hologic Panther Fusion SARS-CoV-2 assay (Fusion) cobas SARS-CoV-2 RT-PCR using cobas 6800 system	Retrospective and prospective	Nasopharyngeal swab (n = 389)	Comparison of 2 platforms and Xpert Xpress SARS-CoV-2 RT-PCR for discrepancy analysis	96.4% agreement in performance	Craney *et al.*[85] 2020
RealTime SARS-CoV-2 assay using *m*2000 system (Abbott)	Validation and verification	Nasal and nasopharyngeal swab (n = 30)	Comparison to CDC SARS-CoV-2 assay	Sensitivity 93% Specificity 100%	Degli-Angeli *et al.*[86] 2020
RealTime SARS-CoV-2 assay using Alinity m system (Abbott) cobas SARS-CoV-2 RT-PCR using cobas 6800 system (Roche)	Prospective	Nasopharyngeal swab (n = 2129)	Clinical evaluation of performance	100% PPA,96.8% NPA	Kogoj *et al.*[87] 2021

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Table 2
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Platform(s) Evaluated	Study Design	Type and Number of Specimens	Comparator Method	Results of Study	Reference
NeuMoDx 96 Molecular System (Ann Arbor, MI)	Retrospective (stored for < 5 d)	Nasopharyngeal swab (*n* = 159)	Comparison of NeuMoDx to Diasorin Simplexa SARS-CoV-2 direct assay and CDC SARS-CoV-2 assay	100% PPA and NPA	Lima *et al.*[88] 2020
NeuMoDx 96 Molecular System (Ann Arbor, MI)	Multicenter comparison, retrospective	Nasopharyngeal swab (*n* = 212)	New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel and RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany)	99% PPA,91.5% NPA	Mostafa *et al.*[89] 2020

Abbreviations: NPA, negative percent agreement; PPA, positive percent agreement.

increase testing capacity while maintaining flexibility to satisfy a wide range of clinical needs. Table 3 summarizes the factors that may be considered by the laboratory before implementing these expensive platforms.

Alternative Technologies for Diagnosis

Diversification of technologies can also aid in the scale up of testing, especially given the concerns with reagent availability and supply chain issues. Several new technologies have been developed during the pandemic that can be implemented in large-scale testing.

CRISPR and associated Cas protein-based diagnostics are powerful methods for nucleic acid detection using cleavage activity. These are typically used in conjunction with reverse transcription loop-mediated isothermal amplification (RT-LAMP) and other isothermal methods. The assays SHERLOCK AND DETECTR have been

Table 3
Factors to consider before acquisition of automated platforms for high-throughput assays during a time of crisis

Goal	Key Parameters to be Assessed	Other Factors
Is this the right assay for this disease?	• Clinical condition being tested • Specimen types or matrix that will be tested • Instrument turnaround time	Clinical and analytical performance characteristics of assay: • Sensitivity • Specificity • Lower limit of detection • Positive and negative predictive values
Is this the appropriate instrument for this test?	• Throughput of instrument (number of tests per 8-h shift) • Hands-on time required before specimen is loaded on the instrument • Availability of reagents, compatibility with commercial reagents	• Batch tested vs random access • Available staffing • Backup plans to mitigate risks for reagent or supply shortages
Is this the appropriate instrument for my laboratory?	• Cost of the instrument and cost per assay • Price of maintenance and repairs • Compatibility with existing testing protocols used in the laboratory • Adaptability to future tests that may be introduced to the laboratory • Instrument footprint	• 5-y return on investment • Service contract costs • Downtime associated with maintenance • Capability to transition laboratory-developed assays to the automated platform • Assessment of assays that are in development for this instrument and whether they fit in with the future plan of the laboratory • Available laboratory space • Need for current or future construction

validated on clinical specimens and are based on cleavage of reporter RNA molecules by the Cas12/13 enzymes.[57] They are available in lateral flow and fluorescence-based readouts and have also been adapted for direct testing of specimens. Further modification of the RNA extraction step by using magnetic beads in conjunction with CRISPR-based assay has been used to expedite detection of SARS-CoV-2 RNA in a "one-pot" test.[58–60] A platform for rapidly scalable diagnostic testing with multiplexing capability has been described by Ackerman and colleagues[61] in the CARMEN-Cas13 assay design. This immensely scalable platform is based on the CRISPR-Cas13 detection system and applied in a combinatorial plate-based format to increase throughput and multiplexing capability.

Nanotechnology is another option that could reduce reagent cost. Use of magnetic nanoparticles for RNA extraction can significantly scale up diagnostic testing and has been advocated for areas with limited resources.[62] The small size and photostability of quantum dots and gold nanoparticles have been used in a colorimetric assay to detect SARS-CoV-2 nucleocapsid gene RNA. Another example is a clinical diagnostic biosensor molecule using gold nanoislands, which can precisely detect selected SARS-CoV-2 sequences in a multigene mixture with low false positive rates.[63,64] Finally, biosensors using graphene–gold nanoparticle platforms can generate an electric readout that was found to be highly sensitive and accurate with rapid turn-around time.[65]

Isothermal amplification techniques such as RT-LAMP have been investigated to ramp up testing as they do not require thermal amplification and therefore the need to transport specimens to a centralized laboratory. They are also amenable to testing crude samples as they are agnostic to PCR inhibitors. Use of multiple primers increases the versatility of these assays in multiplexed reactions. The major disadvantage of the isothermal techniques is the lower sensitivity and specificity when compared with RT-PCR and the requirement for significant optimization for performance comparable to conventional RT-PCR. Several iterations of these assays have been developed in the form of lateral flow or biosensor-based platforms for use in large scale testing at entry points, after addressing the performance characteristics.[66–68]

Although systems like CARMEN have the theoretic potential to perform thousands of assays during a single 8-h shift, most clinical laboratories have been performing the bulk of their testing on commercial automated platforms that use modifications of conventional RT-PCR assays. The newer techniques remain in the research realm because of several challenges and bottlenecks associated with deploying a new assay into a clinical laboratory in the middle of a public health crisis. These include but are not limited to regulatory compliance, complexity of the assays, and adaptability to the CLIA-certified laboratory. Finally, the biggest bottleneck is finding commercial partners such that the reagent and consumable supply chain can be maintained as long as enhanced testing capacity is required.

Next-Generation Sequencing Large-Scale Surveillance of SARS-CoV-2

The need to understand the route of transmission, phylogeny, and molecular evolution of the virus was appreciated early in the pandemic. The emergence of more transmissible variants of SARS-CoV-2 or those that evade immunity induced by vaccines have prompted the development of novel therapeutics. The changing landscape of viral variants underscore the need to monitor their evolution in real-time.[69] Global surveillance efforts (such as COGUK)[70] and sharing of genome sequences in publicly available databases (GISAID)[71] has made an immense impact in efforts to understand the evolution and spread of the viral mutants as well as in studying the immune response to

vaccines.[72] Several commercial assays based on NGS have been adapted to high-throughput formats. These platforms can provide comprehensive information about viral genomes for thousands of individuals in a single run.[73,74] Another important role played by NGS is the monitoring and surveillance of environmental samples, such as wastewater for SARS-CoV-2.[75] Levels of viral RNA in these samples have been shown to increase and decrease ahead of case counts making their monitoring useful for early warning systems, including for the detection of variants of concern.[76] Rapid, multiplexed RT-PCR based assays that detect mutations defining variants of concern have been described for both surveillance and screening.[77–79]

LIMITATIONS OF MOLECULAR ASSAYS AND FUTURE STRATEGIES FOR LARGE-SCALE TESTING

As newer technologies and innovative platforms are introduced to laboratories worldwide, strategies must be developed to expeditiously remove the bottlenecks of

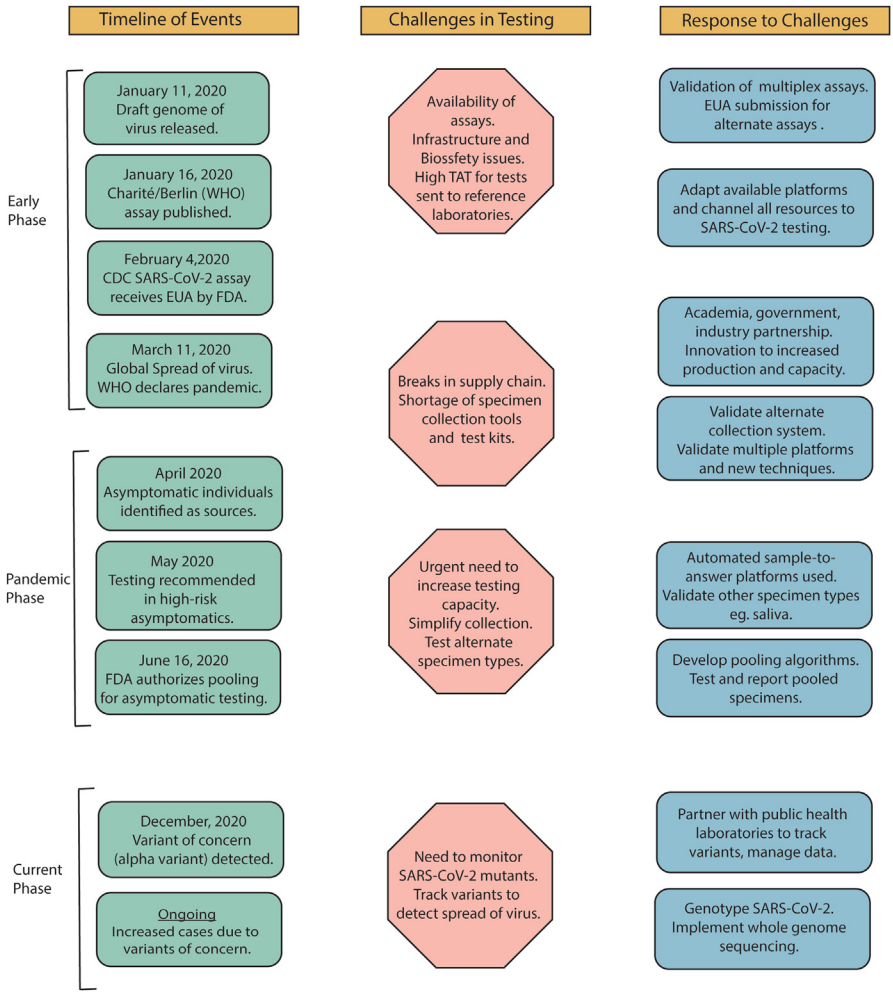

Fig. 2. Timeline and challenges of high-capacity testing. TAT, Turnaround time.

standardization and validation globally. Maintenance of quality control of reagents will be important in deployment of assays. Industry partnership and collaboration with local regulatory authorities will need to be planned.[80] Most importantly, continuous maintenance of the global supply chain is needed to sustain testing capacity.

Another important limitation is that the performance of RT-PCR and other molecular assays have been variable based on the specimen source. Early in the pandemic, lower respiratory swab specimens were reported to be more sensitive in detecting low viral copy numbers than upper respiratory tract specimens.[35] Alternate specimen types such as saliva and oropharyngeal swabs were extremely useful in diagnostic and surveillance testing whereas stool, urine, and blood were not deemed to have sufficient sensitivity to be of use. This variation of detectable RNA quantity in specimen sources will continue to affect the sensitivity and specificity of assays that are being developed. Carefully done studies comparing test performance by body site of collection using standardized gold standards are essential for informing testing algorithms and will become increasingly important if a virus evolves to have new tissue tropism. In addition, RT-PCR assays do not provide essential information regarding viability of the virus.

As with many molecular assays for RNA viruses, continuous monitoring of the performance of primers and probes is required as mutations accrue because of the natural evolution of the virus in response to immune selective pressures and other forces. Significant mutations in the primer/probe binding sites can alter the performance of an assay, thus affecting diagnosis and control efforts. FDA monitors SARS-CoV-2 mutations for possible impact on assay performance,[81] but clinical laboratories are often the first place that changes in analytical performance are noted because of their close involvement with clinicians treating patients with COVID-19. The need to scale up genomic surveillance to detect viral mutants will continue to remain a challenge in the near future.[82] A summary of the challenges of high-capacity testing is shown in Fig. 2.

SUMMARY

Clinical laboratories have stepped up to the unprecedented challenges brought on by the COVID-19 pandemic. Although sufficient testing was not available during the initial weeks of the pandemic, multiple strategies were successfully used to address the challenge of extremely high-capacity testing with reliable results. Although many approaches to pooling were proposed, the simple Dorfman algorithm of combining 4 to 10 original specimens before extraction is the most frequently used. Traditional RT-PCR platforms evolved from low-throughput laboratory developed assays to emergency-use authorized commercial assays on high-throughput platforms. However, the diversification of platforms only partially alleviated the supply shortages that persisted for many months. These challenges spurred the development of many innovative technologies such as highly multiplexed CRISPR-based assays although these remain largely in the research and public health realm. As the virus continually evolves, clinical laboratories must remain vigilant and work closely with state and federal public health agencies to ensure the fidelity of their large-scale testing algorithms and platforms remains intact.

CLINICS CARE POINTS

- Multiple strategies for pooling of different types specimens provided sensitive and specific SARS-CoV-2 testing.

- Automated liquid handling, alternative extraction procedures, multiplexing, and rapid commercialization of new testing platforms added to overall testing capacity.
- Innovation of molecular methods, such as CRISPR-based assays, diversified options for testing and also increased overall testing capacity.

DISCLOSURE

The authors have nothing to disclose.

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