Evaluation of automated molecular tests for the detection of SARS-CoV-2 in pooled nasopharyngeal and saliva specimens

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Abstract

Background: Pooling of samples for SARS-CoV-2 testing in low-prevalence settings has been used as an effective strategy to expand testing capacity and mitigate challenges with the shortage of supplies. We evaluated two automated molecular test systems for the detection of SARS-CoV-2 RNA in pooled specimens.

Methods: Pooled nasopharyngeal and saliva specimens were tested by Qiagen QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAstat) or Cepheid Xpert Xpress SARS-CoV-2 (Xpert), and the results were compared to that of standard RT-qPCR tests without pooling.

Results: In nasopharyngeal specimens, the sensitivity/specificity of the pool testing approach, with 5 and 10 specimens per pool, were 77%/100% (n = 105) and 74.1%/100% (n = 260) by QIAstat, and 97.1%/100% (n = 250) and 100%/99.5% (n = 200) by Xpert, respectively. Pool testing of saliva (10 specimens per pool; n = 150) by Xpert resulted in 87.5% sensitivity and 99.3% specificity compared to individual tests. Pool size of 5 or 10 specimens did not significantly affect the difference of RT-qPCR cycle threshold (C_T) from standard testing. RT-qPCR C_T values obtained with pool testing by both QIAstat and Xpert were positively correlated with that of individual testing (Pearson's correlation coefficient r = 0.85 to 0.99, p < 0.05). However, the C_T values from Xpert were significantly stronger (p < 0.01, paired t test) than that of QIAstat in a subset of SARS-CoV-2 positive specimens, with mean differences of −4.3 ± 2.43 and −4.6 ± 2 for individual and pooled tests, respectively.

Conclusion: Our results suggest that Xpert SARS-CoV-2 can be utilized for pooled sample testing for COVID-19 screening in low-prevalence settings providing significant cost savings and improving throughput without affecting test quality.

Keywords
COVID-19, QIAstat-Dx Respiratory SARS-CoV-2 Panel, sample pooling, SARS-CoV-2, Xpert Xpress SARS-CoV-2
**1 | INTRODUCTION**

Rapid testing to detect individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is central to the management of the ongoing pandemic of coronavirus disease 2019 (COVID-19).\(^1\) Since the beginning of the outbreak, detection of viral RNA in nasopharyngeal (NP) swab specimens by real-time reverse transcription PCR (RT-qPCR) remains the main approach for identifying patients with acute infections.\(^2\) The choice of a method for SARS-CoV-2 RT-qPCR depends on multiple factors including required test throughput, rate of positivity, and the availability of resources. Predesigned assays recommended by the World Health Organization (WHO), targeting several SARS-CoV-2 genes, were made public to enable the development of relatively inexpensive, laboratory-developed RT-qPCR tests early in the outbreak. Many commercial assays have also been developed in singleplex or multiplex formats to test for SARS-CoV-2 RNA. These tests are designed for high-complexity laboratories that perform large volume testing. However, these tests may be difficult to implement in laboratories with limited expertise in molecular testing. To this end, molecular testing devices that integrate RNA extraction and RT-qPCR with random-access features with sample-to-result capability are ideally suited for laboratories that may not be able to deal with high-complexity molecular testing. These test methods and platforms are also suitable for testing in a near point-of-care (POC) setting.\(^3\)

QiAstat-Dx Respiratory SARS-CoV-2 Panel (QiAstat) and Cepheid Xpert Xpress SARS-CoV-2 (Xpert) tests are among the few fully automated, near POC solutions for COVID-19 testing that are FDA approved under emergency use authorization (EUA). While Xpert detects multiple targets within the SARS-CoV-2 genome, QiAstat is a multiplexed RT-qPCR test for the detection of multiple respiratory pathogens, including the SARS-CoV-2 virus in nasopharyngeal specimens.\(^3\) Although these tests are rapid and convenient, they are more expensive than laboratory-developed assays and have lower throughput as each instrument module can only test one sample at a time. The throughput of tests can be improved by using multiple systems or by increasing the number of modules. The GeneXpert systems are available in 1-16 module configurations, and the larger GeneXpert Infinity systems offer a maximum throughput of >2,300 tests per day. However, GeneXpert modules are expensive and significant capital investment is necessary to achieve such test capacity. Pooling of multiple specimens, in low-prevalence settings, may significantly reduce the cost of the commercial, automated molecular tests and improve the throughput of these assays. Furthermore, in the face of massive surges in demand and shortages of test reagents and kits,\(^7\)\(^8\) pooling of samples for COVID-19 testing will help laboratories perform a larger number of tests with limited test kits.

Pooling of specimens to reduce cost and expand the capacity of COVID-19 testing has been well described in the literature.\(^9\)\(^-\)\(^14\) Pooled sample testing was also encouraged by FDA for the screening of asymptomatic individuals for COVID-19.\(^15\) While the pooled sample testing approach was evaluated using laboratory-developed, standard RT-qPCR tests, data on pool testing with automated molecular tests systems such as QiAstat and Xpert are limited.\(^16\)\(^,\)\(^17\) In this study, we evaluated and compared pool testing approach using QiAstat and Xpert on both nasopharyngeal and saliva specimens. Pool test results were verified by individual tests by a WHO-recommended, standard RT-qPCR assay. For a subset of specimens, pool results were also compared to individual test results by the respective commercial tests.

**2 | MATERIALS AND METHODS**

Evaluation of Xpert and QiAstat was performed in the Molecular Infectious Diseases Laboratory of Sidra Medicine, a 400-bed women’s and children’s tertiary care hospital in Qatar, which was designated as a COVID-19-free facility, as part of an integrated, national pandemic management plan. Active screening of patients for COVID-19 was started on March 05, 2020, and the rate of PCR positivity for SARS-CoV-2 has remained <3% of the submitted samples. Standard COVID-19 testing in our laboratory involves extraction of viral RNA from nasopharyngeal flocked swab (NPFS) (BD) or saliva specimens in automated nucleic acid extraction platform NucliSSENS EasyMAG (bioMerieux) followed by RT-qPCR, based on one of the assays recommended by the WHO.\(^18\) The performance standards of the standard method were established in our laboratory according to College of American Pathologists (CAP) guidelines. By using quantitative synthetic SARS-CoV-2 RNA control (Twist Bioscience, CA), the limit of detection of the assay was estimated to be <10 copies/reaction. By testing external quality assessment (EQA) specimens (CAP and Quality Control for Molecular Diagnostics, QCMD), and specimens previously tested in a reference laboratory, the accuracy of the assay is >95%. Both QiAstat and Xpert are fully automated, multiplex real-time RT-PCR tests. While QiAstat is intended to detect multiple respiratory pathogens, including SARS-CoV-2, Xpert is designed to detect the E and N genes of SARS-CoV-2. A total of 815 NPFS and 150 saliva specimens were simultaneously assessed by the standard approach and one of the pool testing approaches in this study. For pool testing, 0.1 ml of each of the 5 or 10 specimens was pooled together, vortexed for 10 s, and 0.3 ml of pooled specimen was analyzed by Xpert or QiAstat according to manufacturer’s instructions. For a subset of samples (n = 60), pool test results were also verified by individual tests using respective commercial assays.

To compare SARS-CoV-2 RT-qPCR C\(_T\) values from different assays, only the C\(_T\) values for the E gene were compared. The linear correlation of RT-qPCR C\(_T\) values obtained by pool testing and individual testing was evaluated by measuring Pearson’s correlation coefficient (r) and associated p-values. The statistical significance of differences in C\(_T\) values between pool testing by QiAstat or Xpert and individual standard testing was calculated by the Mann-Whitney U test. The statistical significance of differences in C\(_T\) values on the same specimens tested by individual and pool testing approaches by QiAstat and Xpert was calculated by paired, Student’s t test. All
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statistical analyses were performed in GraphPad Prism 9.0. The study involves laboratory validation of test methods and the secondary use of anonymous, residual pathological specimens that falls under the category “exempted” by the Sidra Medicine Institutional Review Board.

3 RESULTS

3.1 Pool testing of nasopharyngeal specimens by QIAstat for detection of SARS-CoV-2

Nasopharyngeal flocked swab specimens were tested by QIAstat either in a pool of 5 specimens (pool-5) or in a pool of 10 specimens (pool-10) (Table 1). For pool-5 testing, a total of 10 pooled runs (n = 50 specimens) that gave positive results and 11 pooled runs (n = 55 specimens) that gave negative results by QIAstat were individually assessed by standard RT-qPCR. For pool-10 testing, specimens in 14 positive pools (n = 140 specimens) and 12 negative pools (n = 120 specimens) were individually assessed. QIAstat test results for respiratory pathogens other than SARS-CoV-2 were disregarded. A total of 10 positive pools were missed by the QIAstat pool testing approach: The RT-qPCR C<sub>T</sub> values for these specimens ranged from 32.6 to 38.3. To investigate whether lower analytical sensitivity of QIAstat was responsible for these discrepant results, we individually tested some of these specimens by Xpert and noted that the positive samples, that were missed by QIAstat, were also negative by individual Xpert testing, although they were positive by two other methods (Table S2). The mean C<sub>T</sub> values for these samples by standard RT-qPCR and Xpert were 35.1 ± 1.5 and 35.1 ± 1.9, respectively. For samples that were positive by both pool testing and individual testing approach, the C<sub>T</sub> values were positively correlated (Pearson’s correlation coefficient r = 0.9719 and 0.981; p < 0.001; for pool-5 and pool-10, respectively) (Figure 1A, B). Mean C<sub>T</sub> value change (ΔC<sub>T</sub>) because of pooling 5 and 10 specimens was not significantly different from each other (p = 0.5354) (Figure 1F).

For pools testing of NP swabs by Xpert, a total of 31 pooled runs (n = 155 specimens) that gave positive results and 19 pooled runs (n = 95 specimens) that gave negative results by Xpert were individually assessed by standard RT-qPCR. For pool-5 testing, a total of 14 positive pools (n = 70 specimens) and 20 negative pools (n = 100 specimens) were individually assessed. For pool-10 testing, a total of 140 specimens were individually assessed. QIAstat test results for respiratory pathogens other than SARS-CoV-2 were disregarded. A total of 12 positive pools were missed by the QIAstat pool testing approach: The RT-qPCR C<sub>T</sub> values for these specimens ranged from 26.4 to 38.3. To investigate whether lower analytical sensitivity of QIAstat was responsible for these discrepant results, we individually tested some of these specimens by Xpert and noted that the positive samples, that were missed by QIAstat, were also negative by individual Xpert testing, although they were positive by two other methods (Table S2). The mean C<sub>T</sub> values for these samples by standard RT-qPCR and Xpert were 35.1 ± 1.5 and 35.1 ± 1.9, respectively. For samples that were positive by both pool testing and individual testing approach, the C<sub>T</sub> values were positively correlated (Pearson’s correlation coefficient r = 0.9719 and 0.981; p < 0.001; for pool-5 and pool-10, respectively) (Figure 1A, B). Mean C<sub>T</sub> value change (ΔC<sub>T</sub>) because of pooling 5 and 10 specimens was not significantly different from each other (p = 0.5354) (Figure 1F).

3.2 Pool testing of nasopharyngeal specimens by Xpert for detection of SARS-CoV-2

For pools testing of NP swabs by Xpert, a total of 31 pooled runs (n = 155 specimens) that gave positive results and 19 pooled runs (n = 95 specimens) that gave negative results by Xpert were individually assessed by standard RT-qPCR.
FIGURE 1 Correlation between RT-qPCR $C_T$ values obtained by pooled testing and standard methods. $C_T$ values obtained from QIAstat pool-5 (A) and pool-10 testing (B) and Xpert pool-5 (C) and pool-10 testing (D) in NP specimens and Xpert pool-10 testing in saliva specimens (E) were plotted against $C_T$ values obtained by standard testing and fitted in a linear regression model (Pearson's correlation coefficient = $r$). For the positive pools that gave multiple positive results by individual assessment, the lowest (strongest) $C_T$ was used for analysis (Table S1). (F) mean $C_T$ value difference ($\Delta C_T$) between pool $C_T$ and standard individual $C_T$ with 95% confidence intervals (CI). Statistical significance ($p$) of difference of $\Delta C_T$ between different groups was calculated by Mann-Whitney U test.

FIGURE 2 Correlation between $C_T$ values obtained by pooled testing and individual testing by QIAstat and Xpert tests. $C_T$ values obtained from QIAstat pool-10 (A) and Xpert pool-10 (B) were plotted against individual $C_T$ values obtained by respective methods and fitted in a linear regression model (Pearson's correlation coefficient = $r$). $C_T$ values are compared between single versus pool-10 testing by QIAstat and Xpert tests (C). $C_T$ values are compared between QIAstat versus Xpert tests with individual and pool-10 testing (D).
individually assessed by standard RT-qPCR. For pool-10 testing, specimens in 11 positive pools (n = 110 specimens) and 9 negative pools (n = 90 specimens) by Xpert were individually assessed. By individual testing, a total of 4 pools had >1 positive samples in the pool-5 group (Table S1). All pool test results, except one, were correct in each of the pool-5 and pool-10 groups. The sensitivity, specificity, and accuracy of Xpert pool testing in NP specimens against standard, individual testing were >95% in both group and were not affected by pool size of 5 or 10 specimens (Table 1). The samples that gave a false-negative result in pool-5 had a PCR Ct value of 39 by the standard test, and the sample that gave a false-positive result in pool-10 had a PCR Ct value of 42.5 by Xpert. For samples that were positive by both methods, the RT-qPCR Ct values were positively correlated (Pearson's correlation coefficient $r = 0.9719$ and $0.9583$; $p < 0.0001$; for pool-5 and pool-10, respectively) (Figure 1C,D). Mean Ct value change ($\Delta$Ct) because of pooling 5 and 10 specimens was not significantly different from each other ($p = 0.4765$) (Figure 1F). However, the mean Ct value difference of QIAstat vs Xpert pool testing ($\Delta$Ct) from standard testing was significantly different ($p < 0.01$) (Figure 1F).

### 3.3 Pool testing of saliva specimens by Xpert for detection of SARS-CoV-2

For testing of pooled saliva specimens by Xpert, a total of 8 pooled runs (n = 80 specimens) that gave positive results and 7 pooled runs (n = 70 specimens) that gave negative results were individually assessed by standard RT-qPCR. All except two (87%) pooled test results were correct. The sensitivity, specificity, and accuracy of saliva pool testing by Xpert against standard, individual testing were 87.5%, 99.3%, and 98.7%, respectively (Table 1). For samples that were positive by both methods, the RT-qPCR Ct values were positively correlated (Pearson's correlation coefficient $r = 0.8535$, $p < 0.05$) (Figure 1E). Mean Ct value change ($\Delta$Ct) because of pooling 10 saliva specimens was not significantly different from pool-10 testing of NP swab specimens ($p = 0.6507$) (Figure 1F).

### 3.4 Correlation and comparison of Ct values by QIAstat and Xpert tests in individual and pooled specimens

For a direct comparison of Ct values, we tested a subset of known positive specimens (n = 6; $C_t < 30$ by standard testing) by both QIAstat and Xpert tests both individually and in a pool of 10 NPFS specimens. The Ct values obtained by individual versus pool testing were positively correlated by both QIAstat ($r = 0.9868$, $p < 0.001$) and Xpert ($r = 0.8631$, $p < 0.05$) testing (Figure 2A,B). The mean Ct value changes ($\Delta$Ct) because of pooling 10 NPFS specimens were $-3.683$ (95% CI, $-5.106$ to $-2.261$) and $-3.333$ (95% CI, $-5.861$ to $-0.8054$) by QIAstat and Xpert, respectively, which was not significantly different from each other ($p = 0.7316$) (Figure 2C). However, Ct values obtained by Xpert were significantly ($p < 0.01$ by paired, Student’s t test) lower or stronger than those of QIAstat tests, with mean difference of $-4.250$ (95% CI, $-6.795$ to $-1.705$) and $-4.6$ (95% CI, $-6.703$ to $2.497$) for individual and pooled tests, respectively (Figure 2D).

### 4 DISCUSSION

QIAstat and Xpert tests are among the commercial rapid multiplex PCR assays for SARS-CoV-2 detection that have recently been independently evaluated and demonstrated to have high sensitivity and specificity against standard RT-qPCR tests.\(^4\)\(^6\) These tests are easy to perform without any specific special skills and are suitable for near POC applications, requiring approximately 30–70 min in instrument run-time. However, the higher test cost makes them less suitable as a routine screening tests. The test equipment is modular, where each module can process only one specimen at a time. In order to improve the throughput of these assay, large number of modules are necessary which requires a larger capital investment. As an alternative solution, we performed pooled specimen testing using these platforms and compared the accuracy of results with that of standard RT-qPCR. Our new pooled test approach demonstrated equivalent results to standard testing, in particular with Xpert test and significantly saved costs. The lower sensitivity of pooled sample run using QIAstat is related to the lower analytical sensitivity of the assay compared to other methods (Figure 2, Table S1) rather than because of sample pooling. For both test platforms, a pool size of 5 or 10 specimens did not significantly affect the sensitivity of the pooled testing approach (Table 1, Figure 1F). In our validation study, we have used fixed numbers of samples in the pools for accurate analytical comparison, but our results suggest that any sample number, up to 10, can be pooled without losing sensitivity of SARS-CoV-2 detection by Xpert test. While pool-10 testing may provide more cost savings than a lower number of samples, smaller pool sizes may help reducing wait time for specimens in low-volume testing laboratories and thus improve the turnaround time. In our real-world clinical practice, we pool 3–10 specimens per test, based on specific cutoff times for specimen receipt, so that the results can be reported within 4 h after receiving the specimens. Because of a lower positivity rate (<2%) of COVID-19 in our patient population, <10% of pooled samples required re-testing. However, in order to avoid re-testing of large numbers of samples, pooled testing should only be implemented in low-prevalence settings and the size of the pools must be adjusted to match the current prevalence in the test population.

Our study has several limitations including a limited number of SARS-CoV-2-positive specimens, and in particular, the number of positive saliva samples was only a few. Additional studies with larger number of samples may be necessary to confirm the findings. Also, the gold standard method used in the validation study is a laboratory-developed test. Although the test method has been developed based on published primer and probe sequences and was...
validated in-house according to CAP guidelines, this test does not have formal regulatory approval.

With the continued rise in COVID-19 cases worldwide and urgent need for easing up lockdown and social distancing measures for economic reasons, the role of COVID-19 screening in asymptomatic individuals has become more important than ever. Therefore, there is a heightened interest in inexpensive and convenient test methods for large volume testing and decentralization of the testing process. Sample pooling for COVID-19 testing has now been recognized by WHO, CDC, and FDA.15,19,20 Automated molecular test platforms such as Cepheid GeneXpert systems are easy to be implemented in any setting, and pooled sample tests in these platforms can reduce the cost by 5- to 10-fold and bring down the per-test cost equivalent to laboratory-developed tests. FDA recommends that pooled tests be in ≥85% percent positive agreement with individual tests. Our results show that Xpert tests can be successfully applied for pooled testing of up to 10 specimens with equivalent clinical sensitivity to that of standard, individual testing, significantly reduce cost and improve the test capacity of new and existing test facilities.

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DATA AVAILABILITY STATEMENT
All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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