QIAstat-Dx Respiratory SARS-CoV-2 Panel Testing in Pooled Nasopharyngeal Specimens for COVID-19 Screening in a Low Prevalence Setting

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Short report

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Abstract

Background: COVID-19 screening in healthcare facilities plays a key role in the management of the ongoing pandemic. Rapid and reliable detection of the virus ensures early identification of cases and targeted measures to prevent transmission of the virus. QIAstat-Dx Respiratory SARS-CoV-2 Panel (QRSP) is a fully automated rapid multiplex PCR assay for common respiratory pathogens including SARS-CoV-2 that can provide sample to result in 70 minutes. However, these tests are less suitable as screening tests because of their high cost and lower throughput.

Objective: In this study, we evaluated the performance of QRSP on pooled nasopharyngeal specimens to reduce the cost and improve the turn-around time (TAT) for reporting negative COVID-19 results in a low prevalence setting.

Methods: Nasopharyngeal (NP) specimens were simultaneously tested by pooled QRSP (~10 specimens/pool) approach and by standard RT-qPCR, and the results were compared. TAT of reporting negative results with pooled QRSP tests were compared to that of standard testing.

Results: In 208 specimens, QRSP test results with specimen pooling were in 96% agreement (Kappa=0.92; 95%CI= 0.75-1) with standard RT-qPCR. Despite pooling, C_T values obtained with QRSP were correlated with that of standard RT-qPCR (Pearson correlation coefficient r=0.8343, p=0.0027). The median TAT for negative COVID-19 results by QRSP pooled approach was 2.8 hours (n=1305) compared to 5.4 hours by standard methods (n=4471).

Conclusion: Pooled QRSP testing can be implemented for COVID-19 screening in low prevalence settings providing significant cost savings and improving TAT without affecting test quality.

Introduction

Rapid testing to detect individuals infected with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) is central to the management of 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 sample throughput, rate of positivity and the availability of resources. The World Health Organization (WHO) recommended pre-designed assays, targeting several SARS-CoV-2 genes, were made available for public use to enable the development of relatively inexpensive, laboratory-developed RT-qPCR tests. Typically, viral RNA from NP swabs is extracted and subjected to a screening PCR followed by confirmatory PCR of positive samples. 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 have long turn-around time (TAT), and as such, pose problems when decisions about patient management and infection

control are urgently required. Molecular testing devices that integrate RNA extraction and RT-qPCR with random-access features can significantly improve the TAT of COVID-19 testing (3).

QIAstat-Dx Respiratory SARS-CoV-2 Panel (QRSP) is a convenient, rapid and a fully automated solution for the detection of SARS-CoV-2 in near point-of-care (POC) settings. QRSP is a multiplexed RT-qPCR test for the detection of multiple respiratory pathogens, including the SARS-CoV-2 virus in nasopharyngeal specimens (4). While the test has the additional advantage of simultaneously detecting other respiratory viruses along with SARS-CoV-2, the test is more expensive than laboratory-developed assays and has low throughput as each machine can only test one sample at a time. To this end, the pooling of multiple specimens, in low prevalence settings, may significantly reduce the cost of the test and help report the negative results much faster, if the rate of positivity is low. In this study, we assessed whether pooling of up to 10 specimens affects the sensitivity of the test. QRSP pooled test results on 208 specimens were verified by individual tests by a WHO recommended, standard RT-qPCR. Our results suggest that positive and negative pool results by QRSP were in 95% agreement with that of standard method. Furthermore, we show that the use of QRSP pooled runs reduced the TAT of negative test reports by half.

Material And Methods

Evaluation of QRSP was performed in the Molecular Infectious Diseases Laboratory of Sidra Medicine, a 400-bed women's and children's 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 in March 05, 2020. Standard COVID-19 testing in our laboratory involves extraction of viral RNA from nasopharyngeal flocked swab (NPFS)(BD) specimens in an automated nucleic acid extraction platform NucliSENS EasyMAG (bioMerieux) followed by RT-qPCR, based on one of the assays recommended by WHO (5). The performance standards of the standard method were established in our laboratory according to College of American Pathologists (CAP) guidelines. For QRSP testing, 0.1 ml of each of the 10 specimens were pooled together, vortexed for 10 sec and 0.3 ml of pooled specimen was analyzed by QRSP according to manufacturer's instructions (Qiagen).

Results

Comparison of pooled QRSP tests with Standard SARS-CoV-2 RT-qPCR:

At the time of introduction of QIAstat-Dx for COVID-19 testing, on April 18, 2020, the prevalence of COVID-19 in our patient population was 0.44%. After initial verification of the QRSP assay, a total of 10 pooled runs (n = 105 specimens) that gave positive results and 14 pooled runs (n = 103 specimens) that gave negative results were individually assessed by standard RT-qPCR. Positive pool results were in 100% agreement with the standard assay. SARS-CoV-2 RNA was weakly (C_T > 38) detected in only one of the 103 specimens that were tested negative by QRSP pool testing approach. On the other hand, at least one

specimen was found to be positive in each of the positive pools (Table 1). Overall agreement of QRSP pool results with standard RT-qPCR was 95% (Kappa = 0.9; 95%CI = 0.7-1.0). Also, the RT-qPCR C_T values obtained by pooled QRSP test were positively correlated (Pearson correlation coefficient r = 0.8343, p = 0.0027) to that of standard RT-qPCR (Fig. 1).

Table 1
Comparison of pooled QRSP test results with individual standard RT-qPCR test results

	Positive pool	Negative pool
No. of specimens	105	103
No. of pools	10	14
No. of specimens/pool	10–13	3–10
No. of positive specimens	19	1
No. of negative specimens	86	102
No. of pools in which 1 specimen is positive	4	1
No. of pools in which 2 specimens are positive	3	0
No. of pools in which 3 specimens are positive	3	0
% agreement (as a pool) with standard RT-qPCR	100	93%

Comparison of TAT between pooled QRSP test and standard SARS-CoV-2 RT-qPCR test:

To compare TAT from the time samples were received in the laboratory to the time results were reported, data on TAT, test date and test results were extracted from laboratory records in Cerner Millennium using Discern Explorer. Between March 05, 2020 to June 14, 2020, a total of 1327 specimens were tested in 158 pools by QRSP and a total of 4611 specimens were tested by standard RT-qPCR. Samples were batched by cut-off time for received samples at 8 am, 2 pm and 8 pm. For QRSP tests, 10 specimens were pooled per test in most cases. However, lesser number of specimens were pooled if < 10 specimens were received by the cut-off time or when urgent requests were received. QRSP test results for respiratory pathogens other than SARS-CoV-2 were disregarded. Negative pool results were reported without delay. Only 8.9% pools were positive. Positive pools were broken and assessed individually by standard RT-qPCR. Median TAT of pool positive (n = 14) and pool negative (n = 1305) test results were 8.3 and 2.8 respectively. On the other hand, median TAT of positive (n = 140) and negative (n = 4471) standard test results were 6.7 and 5.4, respectively.

Discussion

Since the emergence of COVID-19 outbreak, the WHO and many other health authorities around the world have emphasized the critical role of laboratory testing in case management, surveillance and rapid response, and infection prevention and control. In addition to the designated COVID-19 hospitals and testing centers, active surveillance is essential for hospitals that are intended to be kept COVID-19 free to ensure the safety of critical, non-COVID-19 related patients and the hospital staffs involved in the care of these patients. COVID-19 screening in these facilities is also necessary so that any patients, or their companions who test positive, can be immediately transferred to a COVID-19 facility. Rapid molecular tests results are crucial in this setting to reduce patient wait time in the emergency department waiting to be admitted or discharged. Rapid tests are also highly important for patients scheduled for surgery. Although the prevalence of the disease in this setting is very low, hospital laboratories are overwhelmed with the large volume of screening tests that cannot wait.

QRSP is the first commercial rapid multiplex PCR assay for SARS-CoV-2 detection that has recently been independently evaluated and demonstrated to have high sensitivity and specificity against standard RT-qPCR tests. This test is easy to perform without any specific special skills and is suitable for near point-of-care application (4). The test takes approximately 70 min. However, higher test cost makes it less suitable as a routine screening test. The test equipment is modular, where each module can only process one specimen at a time. In order to improve the throughput of the assay, large number of modules are necessary which requires a larger capital investment. As an alternative solution, we performed pooled specimen testing using QRSP and compared the accuracy of results with that of standard RT-qPCR. Our new pooled QSRP approach demonstrated equivalent results to standard testing and significantly saved costs and improved the turn-around time for reporting of negative COVID-19 test results. In our setting, only < 10% of pooled samples needed re-testing, with most samples being tested in pools of 10 specimens. In other settings, the number of specimens to be pooled can be adjusted based on prevalence. However, in order to avoid re-testing of large numbers of samples, pooled QRSP testing should only be implemented in low-prevalence setting.

Conclusions

In conclusion, we evaluated a modified approach for COVID-19 testing by QRSP assay. We demonstrate that pooling of up to 10 specimens does not significantly affect the sensitivity of the assay, compared to standard RT-qPCR, but improve the TAT of negative test reporting. In order to save cost, and improve throughput and TAT of negative test results, pooled QRSP testing approach can be utilized for COVID-19 screening in low prevalence setting, in particular for screening in COVID-19-free hospitals or in health care facilities in countries where the number of cases are low or declining.

Abbreviations

SARS-CoV-2
Severe Acute Respiratory Syndrome coronavirus 2
COVID-19

coronavirus disease 2019
QRSP
QIAstat-Dx Respiratory SARS-CoV-2 Panel
NP
nasopharyngeal
RT-qPCR
reverse transcriptase quantitative PCR
WHO
World Health Organization
TAT
turn-around time
POC
point-of-care
NPFS
nasopharyngeal flocked swab
CAP
College of American Pathologists

Declarations

Ethics approval and consent to participate:

The study involves laboratory validation of test methods and the secondary use of anonymous, residual pathological specimens that falls under the category ‘exempted’ by Sidra Medicine Institutional Review Board.

Consent for publication:

Not applicable. The manuscript does not contain any individual person’s data in any form (including individual details, images or videos).

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions:

MRH analyzed data and wrote the manuscript; MRH and HAH designed the study; HAH, FM, AAH and MNA performed laboratory work; PT provided valuable suggestions and revised the manuscript.

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Figures

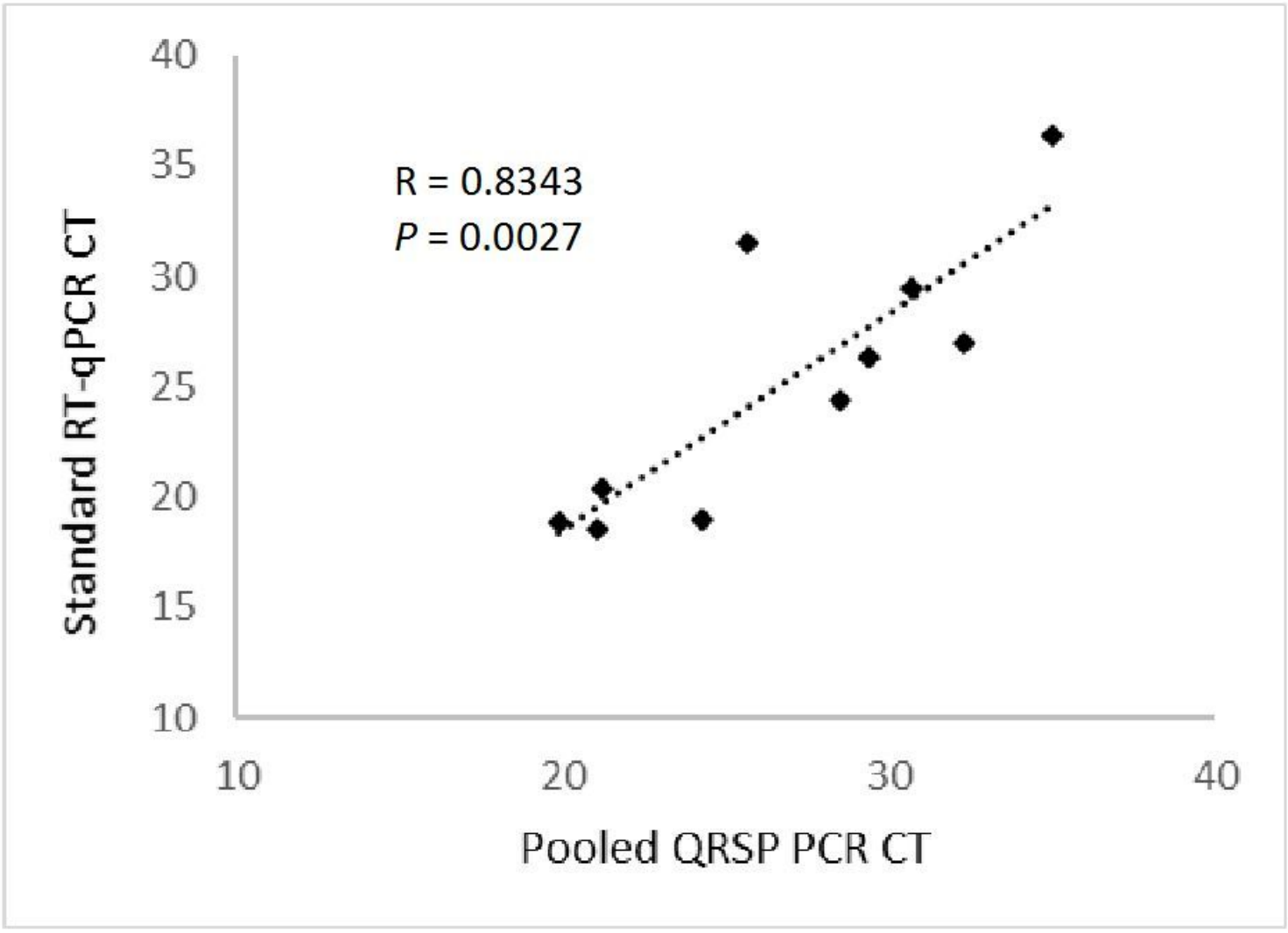

Figure 1

Correlation of RT-qPCR CT values obtained by QRSP and standard methods. CT values obtained by both methods were plotted against each other and fitted in a linear regression model (Pearson correlation coefficient r=0.8343, p=0.0027). For the positive pools that gave multiple positive results by individual assessment, the lowest (strongest) CT was used for analysis.