Impact of Pool Testing in Detection of Asymptomatic Patients with COVID-19

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ABSTRACT

Objective: During the current pandemic, COVID-19 has been detected in patients using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) that confirms the presence of SARS-CoV-2 RNA. The demand for increased testing, particularly for asymptomatic individuals required alternative approaches to single-patient RT-PCR testing, such as pooling.

Methods: This study explored the impact of dilution on the detectability of SARS-CoV-2 in asymptomatic patients using RT-PCR and demonstrated that pooling can be effective in low prevalence populations.

Results: The RT-PCR results for the 3:1, 5:1, and 7:1 aliquot samples showed little differences in CT values, confirming detection capability at these dilutions.

Conclusion: Based on the results of the present study, a pooled approach with up to 5:1 sample aliquots and using the current RT-PCR methodology likely will detect SARS CoV2 RNA among asymptomatic patients.

Keywords: COVID-19, PCR, pooling, RT-PCR, asymptomatic, pandemic

During the current pandemic, COVID-19 has been detected in patients using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) that confirms the presence of SARS-CoV-2 RNA. The escalation of COVID-19 infection numbers in the United States has created a commensurate demand for increased testing, particularly for asymptomatic individuals. Unfortunately, the availability of testing reagents for SARS-CoV-2 RT-PCR has lagged behind the demand; thus, alternative approaches to single-patient RT-PCR testing, such as pooling, are being explored. Of concern for testing asymptomatic patients is whether viral shedding is strong enough for detection using a pooled approach. This study explored the impact of dilution on the detectability of SARS-CoV-2 in asymptomatic patients using RT-PCR.

Materials and Methods

Upon approval from the Institutional Review Board of the Baptist Memorial Hospital system (Memphis, TN), the nasopharyngeal specimens of 9 patients who tested positive for SARS-CoV-2 using single-specimen RT-PCR and who had been clinically determined by a medical professional to be asymptomatic were retrieved from a bank of frozen specimens (–70°C). Each nasopharyngeal swab collected had been placed in a viral transport medium and sent to the laboratory for testing. The specimens were initially tested with the Roche Cobas 8800 using the Roche SARS-CoV2-RT-PCDR kit (Roche Diagnostics, Indianapolis, IN). After the positive results manifested, the specimens had been frozen at −70°C in a specimen bank. For the pooled testing, each specimen was thawed to room temperature and sequentially diluted to create 3:1, 5:1, and 7:1 aliquot specimens, which were then placed in a sterile viral transport medium.
The 3:1 aliquot used a ratio of 400 µL of specimen to 800 µL of transport medium, the 5:1 aliquot used 200 µL of specimen to 800 µL of transport medium, and the 7:1 aliquot used 100 µL of specimen to 600 µL of viral transport medium. The aliquot specimens were tested immediately on the Roche Cobas 8800, and the retrospectively collected threshold cycle (Ct) values were evaluated. Because of the limited specimens available, specimens 6, 7, 8, and 9 were only tested at a 5:1 dilution.

| Patient | Dilution | Ct1   | Ct2   |
|---------|----------|-------|-------|
| 1       | 3:1      | 32.16 | 34.39 |
|         | 5:1      | 32.2  | 34.12 |
|         | 7:1      | 32.23 | 34.29 |
| 2       | 3:1      | 22.01 | 22.43 |
|         | 5:1      | 22.55 | 22.81 |
|         | 7:1      | 22.96 | 23.34 |
| 3       | 3:1      | 22.42 | 23.04 |
|         | 5:1      | 23.38 | 24.05 |
|         | 7:1      | 23.4  | 23.88 |
| 4       | 3:1      | 21.69 | 22.37 |
|         | 5:1      | 22.0  | 22.65 |
|         | 7:1      | 22.15 | 22.82 |
| 5       | 5:1      | 28.43 | 28.65 |
|         | 7:1      | 29.92 | 30.17 |
| 6       | 5:1 only | 24.98 | 25.71 |
| 7       | 5:1 only | 32.35 | 32.86 |
| 8       | 5:1 only | 29.09 | 29.46 |
| 9       | 5:1 only | 32.35 | 32.86 |

Ct, threshold cycle; EUA, emergency use authorization; FDA, U.S. Food & Drug Administration.
Pool dilution for routine use in populations with low prevalence was submitted to the FDA for EUA purposes at a 5:1.

The 3:1 aliquot used a ratio of 400 µL of specimen to 800 µL of transport medium, the 5:1 aliquot used 200 µL of specimen to 800 µL of transport medium, and the 7:1 aliquot used 100 µL of specimen to 600 µL of viral transport medium. The aliquot specimens were tested immediately on the Roche Cobas 8800, and the retrospectively collected threshold cycle (Ct) values were evaluated. Because of the limited specimens available, specimens 6, 7, 8, and 9 were only tested at a 5:1 dilution.

Results

The aliquot testing on all 9 asymptomatic patients confirmed positive results for RT-PCR for COVID-19 at a 5:1 dilution. The Ct value for a positive interpretation is <40. The 9 asymptomatic patients had a variation in Ct values that may reflect differences in viral load (see Table 1). The RT-PCR results for the 3:1, 5:1, and 7:1 aliquot specimens showed little differences in Ct values, confirming the detection capability at these dilutions. In addition, the data suggest that the RT-PCR detection capability is not linear in the dilutions.

Discussion

During a pandemic, the reduced availability of testing reagents and the need to provide testing to a large population necessitate the consideration of pooled testing. The results of this study support the assertion of Cherif et al2 that a pooled approach is more than adequate for COVID-19 testing when individual specimen testing capacity is limited. With pooled testing, however, care must be taken to avoid false negative results incident to overdilution. In addition, a major concern for any type of population testing is how the prevalence of disease in a local region may impact the efficacy of the testing methodology. For example, testing a population with a low prevalence of infection may miss asymptomatic carriers, allowing the wider proliferation of the virus.

Conclusion

Based on the results of the present study, a pooled approach with up to 5:1 specimen aliquots and using the current RT-PCR methodology will likely detect SARS-CoV-2 RNA among asymptomatic patients. LM

References

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