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Extraction-Free Methods for the Detection of SARS-CoV-2 by Reverse Transcription-PCR: a Comparison with the Cepheid Xpert Xpress SARS-CoV-2 Assay across Two Medical Centers

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Reverse transcription-PCR (RT-PCR) is the gold standard for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection; however, testing has been complicated by supply shortages and long turnaround times. A major limiting factor early in the coronavirus disease 2019 (COVID-19) pandemic was the lack of ready availability of the reagents for RNA extraction, which remains an expensive and time-consuming part of some testing modalities. Of great interest, extraction-free methods for use with both nasopharyngeal and saliva sources have been investigated in pilot studies (1–6). In this study, we assessed the detection of SARS-CoV-2 in two clinical laboratories from 144 nasopharyngeal specimens, utilizing an extraction-free method and RT-PCR using CDC primers/probe (Fig. 1A). We compared this method (Direct N2) with the widely used Cepheid Xpert Xpress SARS-CoV-2 assay, which has FDA emergency use authorization (EUA).

Both of the laboratories used previously frozen nasopharyngeal swab specimens in viral transport media (BD Universal viral transport media [UTM] at laboratory 1 and Medical Diagnostic Laboratories viral transport media or institution-produced viral transport media [following CDC standard operating procedure {SOP} no. DSR-052-05] at laboratory 2) which had been previously tested using the Cepheid assay described above. Laboratory 1 subjected 100-μl aliquots of 94 specimens to heat inactivation at 95°C for 10 min in a PCR thermocycler. Laboratory 2 subjected 500-μl aliquots of 50 thawed specimens in cryotubes to heat inactivation in an incubator set to 95°C for 10 min. Samples were brought to room temperature before 3 μl was added to 17 μl of master mix (1.5 μl, CDC 2019-nCoV primer/probe; 10.5 μl, nuclease-free water; 5.0 μl, TaqPath 1-Step reverse transcription-quantitative PCR [RT-qPCR] master mix [4×]). Synthetic "nCoVPC" was used for the positive control in laboratory 1, and whole viral SARS-CoV-2 RNA (kind gift from Scott Weaver, University of Texas Medical Branch) was used in laboratory 2. PCR cycling was performed using CDC parameters on a 7500 Fast real-time PCR instrument and SDS software (version 1.4.1; Applied Biosystems). The N2 primers/probe performed better than N1 and exhibited a mean and range of threshold cycle (C_T) values more similar to those determined for the Cepheid N2 target than to those seen with the N3 target (Fig. 1B). The 3-μl sample input volume outperformed all other sample volumes tested (Fig. 1C); it is possible that inhibitory substances impair performance at higher volumes. The mean C_T values from Direct N2 screening were expectedly higher than the corresponding C_T values from the original Cepheid N2 assay (Fig. 1D), although results of comparisons of C_T values across different assays must be interpreted with caution. Most of the specimens with Cepheid N2 C_T values of <35 were detected with Direct N2 (89/91, 98%), whereas Direct N2 performed markedly less well for

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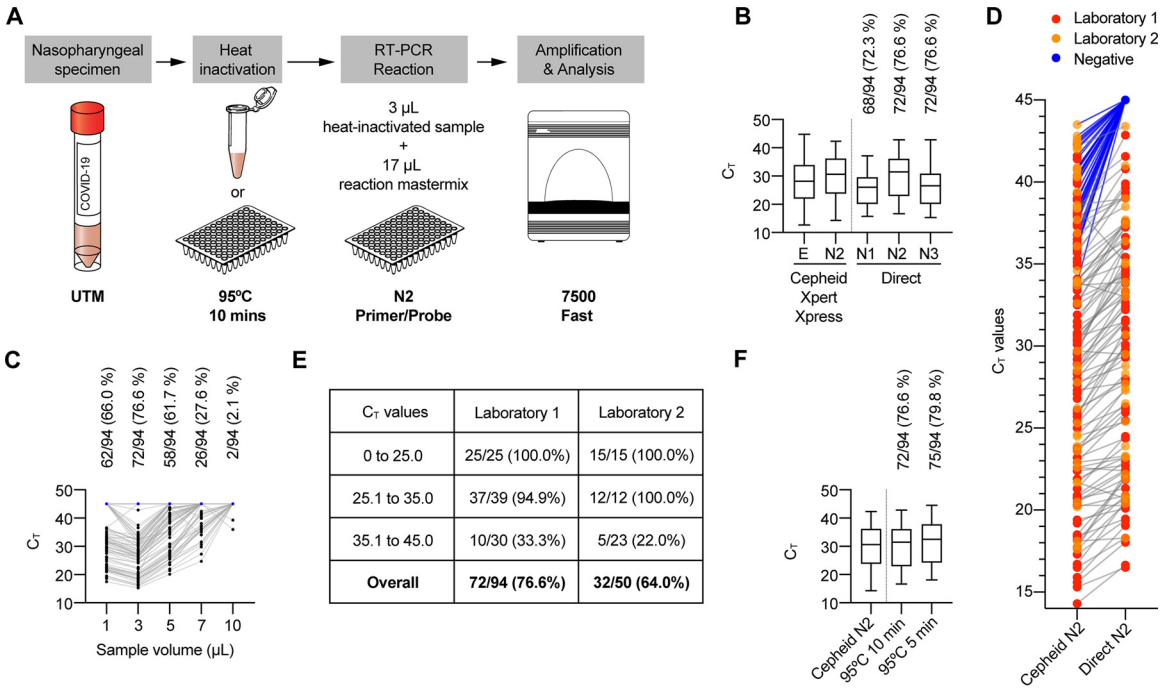

C_T values	Laboratory 1	Laboratory 2
0 to 25.0	25/25 (100.0%)	15/15 (100.0%)
25.1 to 35.0	37/39 (94.9%)	12/12 (100.0%)
35.1 to 45.0	10/30 (33.3%)	5/23 (22.0%)
Overall	**72/94 (76.6%)**	**32/50 (64.0%)**

FIG 1 Extraction-free SARS-CoV-2 N2 screening. (A) Generalized direct PCR procedure. (B) The performance of the N2 primer/probe pair was most comparable to that seen with the Cepheid N2 target. (C) Optimization of sample volume in reaction mixture. (D) Direct N2 C_T values compared to initial Cepheid N2 C_T value (negative = C_T value of 45). (E) Performance of Direct N2 screening by C_T value and performing laboratory. (F) Shorter heat inactivation duration modestly increased performance.

specimens with Cepheid N2 C_T values of >35 (15/53, 28%) (Fig. 1E). Heat inactivating in plates for 5 min instead of 10 min led to a modest improvement in sensitivity (Fig. 1F).

The direct detection method used in this study is simple, economical, and robust, with an approximate hands-on time of 1 h and an instrument run time of ~80 min to result per 96-well plate. Discrepant results occurred for specimens with Cepheid N2 C_T values of approximately >35. Further, C_T values from Cepheid testing were obtained using fresh specimens, whereas Direct N2 was performed after a freeze/thaw cycle, which may have contributed to increased C_T values. Direct N2 also uses a lower specimen volume, which was likely the primary cause of reduced sensitivity. Overall, the process is high throughput as described here and is amenable to automation. In conclusion, while moderately less sensitive than conventional RT-PCR-based SARS-CoV-2 assays, direct methods may represent a viable high-throughput diagnostic approach.

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