DOI: 10.1002/jmv.27039

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Real-life evaluation of a rapid extraction-free SARS-CoV-2 RT-PCR assay (COVID-19 PCR Fast-L) for the diagnosis of COVID-19

To the Editor,

Timely and rapid diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by reverse transcription-polymerase chain reaction (RT-PCR) is paramount to the control of the coronavirus disease 2019 (COVID-19) pandemic. Attempts have been made to shorten molecular testing turnaround by skipping nucleic acid extraction, thus performing RT-PCR directly on heat-treated respiratory specimens. A number of either "in-house"-developed or commercial (i.e., Cepheid Xpert Xpress SARS-CoV-2 assay) RT-PCR or loop-mediated amplification protocols have been developed and found to display clinical sensitivities ranging from 75% to 98% when compared to RT-PCR preceded by viral RNA extraction.[1–9] Here, we conducted a real-life evaluation of the performance of a commercially-available free-extraction RT-PCR, the Ascires COVID-19 PCR Fast-L (Sistemas Genómicos®), which is multiplexed to amplify two conserved sequences within ORF-1ab/1a (FAM and CY5 channels), one of which lies within the RdRP gene (FAM channel) and returns qualitative results in less than 1 h. In this assay, nasopharyngeal (NP) specimens are transferred to 1 ml of transport/extraction buffer containing proteinase K provided by the manufacturer, placed in a dry bath at 60°C for 5 min, then at 98°C for 2 min, and finally on ice for cooling. Target amplification is carried out using the AriaMx Real-Time PCR System (Agilent®), and results are analyzed and interpreted automatically by The AriaMx Software version 1.5. Thermal cycling conditions are shown in the footnote of Table 1. The assay includes an internal heterologous DNA control (HEX probe). According to the manufacturer, the limit of detection (LOD) of the assay is approximately 4000 copies/ml (95% confidence interval [CI]).

This prospective study enrolled 662 patients between November 23 and December 10, 2020, attended at the Emergency Department of the Hospital Clínico Universitario of Valencia (HCU) with clinical suspicion of COVID-19. The study was approved by the HCU INCLIVA Research Ethics Committee. NP specimens were collected by trained nurses and transferred to 1 ml of transport/extraction buffer, as stated above. Samples were immediately delivered to the Microbiology Service of HCU, where they were kept at 4°C until processed. Specimens were split into two aliquots, one of which was processed as per routine by using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kits coupled with the Thermo Scientific™ KingFisher Flex automated instrument followed by RT-PCR employing the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific).[10] According to the manufacturer, the LOD of the assay is 250 copies/ml (for the N gene target). The other aliquot was analyzed by the COVID-19 PCR Fast-L assay within 24 h upon receipt. The SARS-CoV-2 RNA (AMPLIRUN® TOTAL SARS-CoV-2 RNA Control; Vircell S.A) was used as standard material for estimation of viral loads (in copies/ml) in NP.[10]

Out of 662 NP samples, 68 (10.3%) and 582 (87.9%) returned positive and negative results, respectively, by both assays, and 12 (1.8%) yielded discordant results (TaqPath positive/PCR Fast-L negative), thus resulting in an excellent concordance between the assays, with a Kappa index of 0.90 (95% CI: 0.85–0.96). Overall positive percent agreement (PPA) and negative percent agreement across the assays were 85% (95% CI: 75.5%–91.2%) and 100% (95% CI: 99.3%–100%), respectively. As shown in Figure 1, RT-PCR C_t values were significantly lower ($p < 0.001$) in samples returning

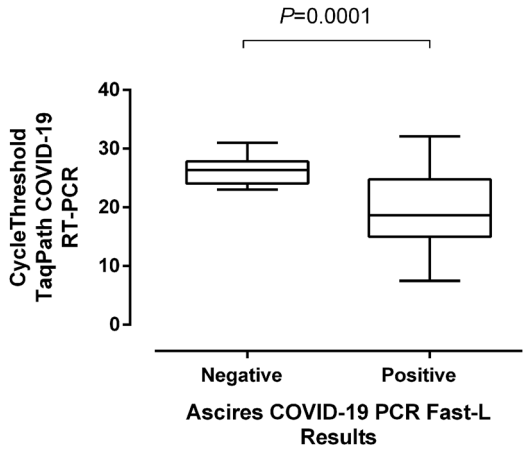

FIGURE 1 SARS-CoV-2 RNA cycle threshold (C_t) RT-PCR value in nasopharyngeal specimens either testing positive by both the COVID-19 PCR Fast-L (Sistemas Genómicos®) and the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific) or returning positive results by the latter assay and negative by the former. *p* Value for the difference between medians is shown and was estimated using the Mann–Whitney *U* test. Statistical analyses were performed using SPSS version 25.0; SPSS. COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

TABLE 1 Performance of the Ascires COVID-19 PCR Fast-L Device in comparison with the TaqPath COVID-19 Combo Kit for the detection of SARS-CoV-2 detection in nasopharyngeal specimens from patients with clinical suspicion of COVID-19 attended at the Emergency Department according to the SARS-CoV-2 viral load

Cycle threshold on comparison RT-PCR (log_{10} copies/ml)[a]	Parameter	
	Positive percent agreement % (95% CI)	Negative percent agreement % (95% CI)
≤22 (≥6.8)	100 (91.8-100)	100 (99.3-100)
≤25 (≥5.9)	94.4 (84.9-98.1)	100 (99.3-100)
≤28 (≥4.9)	85.9 (76-92.2)	100 (99.3-100)
≤31 (≥3.9)	85.7 (76.2-91.8)	100 (99.3-100)
≤34 (≥3.1)	85.0(75.6-91.2)	100 (99.3-100)

Note: In the Ascires COVID-19 PCR Fast-L Kit, a volume of 2.5 µl of treated specimens was added to the amplification plate, previously reconstituted with 17.5 µl of the buffer provided by the manufacturer. Total reactions of 20 µl were obtained by mixing 17.5 µl of master mix (primers and probe mix: ORF1ab and ORF1a) and 2.5 µl of clinical sample to fill the reaction. The thermal cycling steps were: Stage 1, 45°C for 10 min; Stage 2, 95°C for 20 s; Stage 3, 95°C for 5 s, 58°C for 20 s, 40 cycles.

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]The AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Vircell S.A) was used as the reference material for SARS-CoV-2 RNA load quantitation.

positive results by both methods (median C_t: 18.7; range: 7.5–32.1; equivalent to a median of 7.86 log_{10} copies/ml; range: 3.64–11.4) than in those samples displaying discrepant results (median C_t: 26.4; range: 23–31, corresponding to a median of 5.44 log_{10} copies/ml; range: 3.98–6.51). Accordingly, a direct relationship was found between PPA and SARS-CoV-2 RNA load in NP (Table 1). In fact, PPA was maximum (100%) with SARS CoV-2 RNA with loads ≥6.8 log_{10} SARS CoV-2 RNA copies/ml, which roughly corresponds to the viral load threshold above which viable SARS-CoV-2 virus could be recovered from cell culture.[10]

In summary, the free-extraction COVID-19 PCR Fast-L assay, although less sensitive than the comparator RT-PCR used herein, is a reliable test for diagnosis of SARS-CoV-2 infection when NP specimens harbor relatively high viral loads.

ACKNOWLEDGMENTS

The authors are grateful to all personnel who work at Clinic University Hospital, in particular, to staff at the Microbiology laboratory and the Emergency Department for their commitment in the fight against coronavirus disease 2019. Sistemas Genómicos (Grupo Biomédico Ascires, Paterna, Valencia, Spain), had no intervention either in the study design, "real-life" evaluation of the assay or in the interpretation of the data.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Ignacio Torres, Eliseo Albert, Felipe Bueno, Dixie Huntley, and Sandrine Poujois performed the assays and helped in the interpretation of the data. Jamal Qualai and María Teresa Gil developed the COVID-19 PCR Fast-L Kit. David Navarro analyzed and interpreted the data and wrote the manuscript.

Ignacio Torres[1]
Jamal Qualai[2]
Eliseo Albert[1]
Felipe Bueno[1]
Dixie Huntley[1]
Sandrine Poujois[1]
María Teresa Gil[2]
David Navarro[1,3]

[1]*Microbiology Service, Hospital Clínico Universitario, INCLIVA Research Institute, Valencia, Spain*
[2]*Sistemas Genómicos, Grupo Biomédico Ascires, Valencia, Spain*
[3]*Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain*

Correspondence
David Navarro, Microbiology Service, Hospital Clínico Universitario, Instituto de Investigación INCLIVA and Department of Microbiology, University of Valencia, Av. Blasco Ibáñez 17, 46010 Valencia, Spain.
Email: david.navarro@uv.es

Ignacio Torres and Jamal Qualai contributed equally to this study.

ORCID

David Navarro http://orcid.org/0000-0003-3010-4110

REFERENCES

1. Mokhtar KM. Improved COVID-19 testing by extraction-free SARS-CoV-2 RT-PCR. *EJIFCC*. 2020;31:362-368.
2. Genoud V, Stortz M, Waisman A, et al. Extraction-free protocol combining proteinase K and heat inactivation for detection of SARS-CoV-2 by RT-qPCR. *PLoS One*. 2021;16:e0247792.
3. Lübke N, Senff T, Scherger S, et al. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. *J Clin Virol*. 2020;130:104579.
4. Kriegova E, Fillerova R, Kvapil P. Direct-RT-qPCR detection of SARS-CoV-2 without RNA extraction as part of a COVID-19 testing strategy: from sample to result in one hour. *Diagnostics*. 2020;10:605. https://doi.org/10.3390/diagnostics10080605
5. Ulloa S, Bravo C, Parra B, et al. A simple method for SARS-CoV-2 detection by rRT-PCR without the use of a commercial RNA extraction kit. *J Virol Methods*. 2020;285:113960.
6. Israeli O, Beth-Din A, Paran N, et al. Evaluating the efficacy of RT-qPCR SARS-CoV-2 direct approaches in comparison to RNA extraction. *Int J Infect Dis*. 2020;99:352-354.

7. Adams NM, Leelawong M, Benton A, Quinn C, Haselton FR, Schmitz JE. COVID-19 diagnostics for resource-limited settings: evaluation of "unextracted" qRT-PCR. *J Med Virol*. 2021;93:559-563.
8. Mancini F, Barbanti F, Scaturro M, et al. Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing. *Emerg Microbes Infect*. 2020;9:1393-1396.
9. Smyrlaki I, Ekman M, Lentini A, et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nat Commun*. 2020;11:4812.
10. Albert E, Torres I, Bueno F, et al. Field evaluation of a rapid antigen test (Panbio COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. *Clin Microbiol Infect*. 2021;27:472.e7-472.e10.