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Novel automated sample-to-result SARS-CoV-2 laboratory-developed RT-PCR assay for high-throughput testing using LabTurbo AIO 48 system

Ming-Jr Jian [a,1], Hsing-Yi Chung [a,1], Chih-Kai Chang [a], Jung-Chung Lin [b], Kuo-Ming Yeh [b], Sheng-Kang Chiu [b], Yi-Hui Wang [a], Shu-Jung Liao [a], Shih-Yi Li [a], Shan-Shan Hsieh [a], Cherng-Lih Perng [a], Feng-Yee Chang [b,*], Hung-Sheng Shang [a,*]

[a] Division of Clinical Pathology, Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei 11490, Taiwan, Republic of China
[b] Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei 11490, Taiwan, Republic of China

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ABSTRACT

Background and aims: Immediate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for preventing the spread of coronavirus disease 2019 (COVID-19). The LabTurbo AIO 48 system is an automated platform that allows nucleic acid extraction and sample analysis on the same instrument, producing faster results without affecting their accuracy. We aimed to independently evaluate the LabTurbo AIO 48 (all-in-one system) for SARS-CoV-2 detection.

Materials and methods: Comparative limit of detection (LOD) was assessed on both the LabTurbo AIO 48 and current standard detection system based on real-time reverse transcriptase polymerase chain reaction (RT-PCR), using SARS-CoV-2 RNA control. Additional 125 primary clinical samples were assessed using both the protocols in parallel.

Results: The turnaround time from sample to results for 48 samples analyzed on LabTurbo AIO 48 was approximately 2.5 h, whereas that analyzed using the in-house RT-PCR protocol was 4.8 h. LabTurbo AIO 48 also demonstrated higher sensitivity than our reference RT-PCR assay, with a LOD of 9.4 copies/reaction. The overall percentage agreement between both the methods for 125 samples was 100%.

Conclusion: LabTurbo AIO 48 is a robust detection option for SARS-CoV-2, allowing faster results and, consequently, aiding in better control and prevention of COVID-19.

1. Introduction

The novel coronavirus reported in Wuhan, Hubei Province, China, in December 2019, has become a global health crisis [1]. On March 11, 2020, the coronavirus disease 2019 (COVID-19) outbreak was declared as a pandemic by the World Health Organization (WHO) [2]. The etiological agent responsible for this new infection is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3]. Currently, real-time reverse transcriptase polymerase chain reaction (RT-PCR) is considered as a gold standard for the diagnosis of SARS-CoV-2 infection due to high sensitivity of the method [4]. Development of robust and sensitive assays that can identify SARS-CoV-2 is critical for patient care and for implementing public health measures to control the infection from spreading. Several in-house and commercial RT-PCR assays have been developed and validated [5–8], while some still require validation. The LabTurbo AIO 48 system (LabTurbo, Taipei City, Taiwan) is an automated platform in which nucleic acid extraction and real-time RT-PCR are performed on the same instrument. This automated system ensures continuous detection of SARS-CoV-2 by targeting different viral genes. To date, SARS-CoV-2 detection assays using the LabTurbo AIO 48 system have not been reported. However, in the present study, it was evaluated and compared with the standard RT-PCR assay currently recommended by the Taiwan Centers for Disease Control (CDC).

Abbreviations: COVID-19, Coronavirus disease 2019; LOD, Limit of detection; RT-PCR, Reverse transcriptase polymerase chain reaction; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

* Corresponding authors.
E-mail addresses: fychang@ndmctsgh.edu.tw (F.-Y. Chang), iamkeith001@gmail.com (H.-S. Shang).

[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Study design and clinical samples

According to the recommendations of the Taiwan CDC and WHO guidelines, the SARS-CoV-2 screening and confirmatory assays were performed by targeting the envelope (*E*) and RNA-dependent RNA polymerase (*RdRp*) viral genes. In accordance with the protocol suggested by the Taiwan CDC, one-step real-time RT-PCR was performed using the primer and probe sequences designed by Corman et al. [9]. The experimental procedure and interpretation of results have been previously described [10,11]. Briefly, one-step real-time RT-PCR was performed on a Rotor-Gene Q real-time PCR machine (Qiagen, Hilden, Germany). Thermal cycling was performed as follows: reverse transcription at 50 °C for 10 min, followed by 95 °C for 2 min, and 50 cycles at 95 °C for 5 s and 58 °C for 30 s. All positive samples were further validated by the Taiwan CDC central laboratory. We evaluated 125 nasopharyngeal swabs (COPAN's COVID-19 Collection & Transport Kits with Universal Transport Medium or Virus Transport Swabs 147C) from patients suspected of having COVID-19. This study was approved by the Institutional Review Board of the Tri-Service General Hospital (TSGHIRB No. C202005041), registered on March 20, 2020. Informed consent was obtained from patients.

2.2. SARS-CoV-2 assay procedure using LabTurbo AIO 48

Remaining patient samples, which were previously used for clinical testing, were repurposed for the SARS-CoV-2 assay on the LabTurbo AIO 48 system. Primer and probe sequences were designed according to previous studies, with some modifications (Table 1) [5,9,12]. The multiplex one-step real-time RT-PCR assay was designed to target both the *ORF1ab* and *E* viral genes. To ensure quality of sample processing, primers and probes for the human ribonuclease P (*RP*) gene were used as internal controls. The positive control was a diluted viral RNA sample from a COVID-19-positive patient, which was aliquoted and stored at −80 °C (Ct values of 34 ± 2 for each run was considered acceptable). A negative control (specimen from COVID-19-negative subject) and a non-template control (RNase-free water) were included in every total nucleic acid extraction procedure and every RT-PCR run, respectively. A total of 500 μL of input sample was used, according to the manufacturer's recommendations (Supplementary Figure S1). Briefly, total nucleic acid containing viral RNA was extracted from 500 μL of nasopharyngeal supernatant and the RNA was eluted in 60 μL RNase-free water. For the LabTurbo AIO 48 system, a 14 μL reaction was automatically prepared for each sample containing 6 μL RNA, 7 μL 2 × SensiFAST Probe No-ROX One-Step mix (Bioline, London, UK), 400 nM forward and reverse primers, 100 nM probe, 0.14 μL reverse transcriptase, and 0.28 μL of RiboSafe RNase inhibitor (Bioline). The RT-PCR protocol was as follows: reverse transcription at 50 °C for 10 min, followed by 95 °C for 2 min, 50 cycles at 95 °C for 5 s and 58 °C for 30 s. The SARS-CoV-2 assay simultaneously detected the SARS-CoV-2 *E* and *ORF1ab* genes along with the human *RP* gene to monitor the quality of nucleic acid extraction. A result was interpreted as positive or negative by the detection of the *E* and *ORF1ab* genes or by the lack of detection of those genes, respectively. An inconclusive result, which did not meet the above conditions, was recorded and the sample was retested.

2.3. Evaluation of analytical sensitivity of the LabTurbo AIO 48 system

A preliminary sensitivity analysis of the LabTurbo AIO 48 system was performed by evaluating serial dilutions of known positive samples and comparing the results with those obtained by following the Taiwan CDC protocol. The limit of detection (LOD) was determined using an AMPLIRUN SARS-CoV-2 RNA control (Vircell, Granada, Spain) that contained purified RNA of the SARS-CoV-2 genome for absolute quantification.

2.4. Evaluation of specificity

The specificity of the laboratory-developed SARS-CoV-2 assay, using the LabTurbo AIO 48 system, was evaluated against several common upper respiratory tract viruses (influenza A, influenza B, rhinovirus, respiratory syncytial virus, parainfluenza virus, and adenovirus). These positive samples were obtained from viral cultures of the Taiwan CDC viral infection contract laboratory.

2.5. Comparison of clinical performance

To evaluate the clinical performance of the lab-developed SARS-CoV-2 assay at varying viral concentrations, using LabTurbo AIO 48 system, 40 positive specimens were selected to represent the full range of observed Ct values (15–34 cycles). Positive agreement was calculated using the Taiwan CDC protocol as the reference method.

3. Results

3.1. Analytical sensitivity of the LabTurbo AIO 48 SARS-CoV-2 assay

The LOD was determined by testing 5–10 replicates of the positive SARS-CoV-2 RNA control (Vircell, Granada, Spain) that was 2-fold serially diluted to around the expected LOD. Using the LabTurbo AIO 48 platform, LOD obtained from 10 replicate tests was found to be 9.4 copies/reaction for the *E* and *ORF1ab* genes (Table 2).

3.2. Analytical specificity of the LabTurbo AIO 48 SARS-CoV-2 assay

We used samples of known upper respiratory tract viruses, including influenza A, influenza B, rhinovirus, respiratory syncytial virus, parainfluenza virus, and adenovirus, to demonstrate the analytical specificity of the *E* and *ORF1ab* gene assay detecting SARS-CoV-2. Additional undiluted cell culture supernatants were also tested. All the LabTurbo AIO 48 SARS-CoV-2 assays were found to be highly specific for SARS-CoV-2 with no cross-reactivity with other upper respiratory tract viruses (Table 3).

Table 1
Sequences of primers and probes.

Target gene	Primer or probe name	Sequence (5′→3′)	References
ORF1ab	China_CDC_ORF1_F nCoV_N1-R	CCC TGT GGG TTT TAC ACT TAA	[5]
	China_CDC_ORF1_R nCoV_N1-R	ACG ATT GTG CAT CAG CTG A	
	China_CDC_ORF1_P	FAM-CCG TCT GCG /ZEN/ GTA TGT GGA AAG GTT ATG G-3IABkFQ/	
E	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	[9]
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	
	E_Sarbeco_P1	HEX-ACACTAGCC/ZEN/ATCCTTACTGCGCTTCG/3IABkFQ/	
RP	RP-F	AGA TTT GGA CCT GCG AGC G	[12]
	RP-R	GAG CGG CTG TCT CCA CAA GT	
	RP-P	Cy5-TTC TGA CCT GAA GGC TCT GCG CG/31AbRQSp/	

Table 2
Assessment of Limit of detection for SARS-CoV-2 *E* and *ORF1ab* gene assay on the LabTurbo AIO 48 platform.

	Target gene	
Viral load (copies/reaction)	*E* gene	*ORF1ab* gene
4.7	36.97	N.D.
	35.97	37.21
	N.D.[#]	N.D.
	N.D.	N.D.
	N.D.	N.D.
	N.D.	N.D.
	N.D.	N.D.
	N.D.	N.D.
	N.D.	N.D.
	N.D.	N.D.
9.4	33.81	34.32
	33.94	34.47
	33.74	34.58
	34.76	34.92
	35.15	36.81
	34.42	34.41
	34.86	34.64
	34.00	34.48
	33.63	34.45
	34.92	35.75
18.75	32.55	33.75
	32.98	33.72
	32.84	33.74
	32.57	33.47
	32.85	33.39
37.5	30.43	32.79
	31.9	32.02
	31.15	32.57
	32.11	32.16
	31.84	31.83
75	30.75	31.8
	29.51	31.61
	29.74	31.38
	30.27	31.05
	29.82	30.93

[#] N.D., Not Detected.

Table 3
Tests of known respiratory viruses in cell culture preparations for cross-reactivity in SARS-CoV-2 *E* and *ORF1ab* gene assay on LabTurbo AIO 48 platform.

	SARS-CoV-2 RT-PCR	
Clinical viral isolated with known viruses	*E* gene	*ORF1ab* gene
Influenza A(H1N1)	N.D.	N.D.
Influenza A(H3)	N.D.	N.D.
Influenza A(H1)	N.D.	N.D.
Influenza B	N.D.	N.D.
Rhinovirus/enterovirus	N.D.	N.D.
Respiratory syncytial virus	N.D.	N.D.
Parainfluenza 1 virus	N.D.	N.D.
Parainfluenza 2 virus	N.D.	N.D.
Parainfluenza 3 virus	N.D.	N.D.
Adenovirus	N.D.	N.D.

N.D., Not Detected.

3.3. Clinical performance of the LabTurbo AIO 48 SARS-CoV-2 assay

Of the 125 samples collected from patients, 85 and 40 samples were identified as negative and positive for SARS-CoV-2, respectively (Table 4). Both the LabTurbo AIO 48 assay and the Taiwan CDC recommended assay, which used the same primer set for targeting the SARS-CoV-2 *E* gene, showed 100% positive agreement, including for low (Ct value > 30), medium (Ct value 20–30), and high (Ct value < 20) viral load (Table 5). Further analysis of the Ct values of the SARS-CoV-2 *E* gene-positive specimens (n = 40) confirmed that the data obtained from the LabTurbo AIO 48 system highly correlated ($R^2 = 0.9824$) with those obtained from the Taiwan CDC recommended RT-PCR assay (Fig. 1).

4. Discussion

In this study, we described an automated, sample-to-result, real-time RT-PCR assay for the detection of SARS-CoV-2 using the LabTurbo AIO 48 system. This approach was designed to identify two viral genes (*ORF1ab* and *E*) in a single reaction tube. Matheeussen et al. suggested that testing capacity may be the main challenge of the current SARS-CoV-2 pandemic [13], highlighting the urgent need for reliable, high-throughput assays for SARS-CoV-2 detection. In the present study, it was demonstrated that the use of LabTurbo AIO 48 system shortened the turnaround time by approximately 47.9%, without compromising on sensitivity or specificity, while handling 48 samples at one time (Supplementary Figure S2). The LabTurbo AIO 48 system can handle 864 samples/day in a continuous operation mode. Several primer and probe sets targeting different viral genes of SARS-CoV-2 have been developed [14]; however, different RNA extraction methods can also influence the test outcome [14,15]. The LabTurbo AIO 48 system uses a patented "membrane tube vacuum flow extraction technology" to produce high-purity, high-yield total nucleic acid to improve the detection sensitivity. In response to the urgent need for large scale diagnostic testing during the COVID-19 pandemic, several molecular tests have been authorized for emergency use by the US Food and Drug Administration [6,8,16]. Both Cepheid Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) or Abbott ID Now SARS-CoV-2 (Abbott, Chicago, IL) tests offer shorter turnaround times of less than 1 h; however, the single specimen cost is much higher, specific equipment and its corresponding instruments are required, and only one specimen can be loaded at a time. Similarly, Roche's cobas SARS-CoV-2 assay on the 6800 platform (Roche Diagnostics, Indianapolis, IN) provides up to 96 results in about 3 h per workflow, but the cost is high and specific equipment and its corresponding instruments are required. In summary, our LabTurbo AIO 48 platform is high-throughput, cost-effective, and easy to install in other diagnostic laboratories. The sensitivity and specificity of the LabTurbo AIO 48 system and the in-house RT-PCR SARS-CoV-2 assay proved to be 100% concordant with the standard clinical protocol, demonstrating that the LabTurbo AIO 48 system represents a promising commercial alternative for the detection of SARS-CoV-2.

5. Conclusion

High-throughput and reliable SARS-CoV-2 diagnostic tests are critically important during this worldwide pandemic. Our multiplex RT-PCR coupled with the LabTurbo AIO 48 system can provide meaningful information to the clinical staff, as well as assist other laboratories to develop testing protocols for the detection of SARS-CoV-2.

Author Contributions

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Ming-Jr Jian: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **Hsing-Yi Chung:** Formal

Table 4
Comparison of the LabTurbo AIO 48 SARS-CoV-2 assay and the currently recommended Taiwan CDC assay.

Sample case	Taiwan CDC assay			LabTurbo AIO 48 platform assay		
	RdRp gene	*E* gene	Interpretation*	*ORF1ab* gene	*E* gene	Interpretation*
1	23.41	23.45	Positive	24	23.77	Positive
2	30.23	29.73	Positive	31.36	29.71	Positive
3	24.06	23.92	Positive	24.79	24.72	Positive
4	15.91	15.7	Positive	16.9	17.67	Positive
5	26.92	26.62	Positive	29.02	27.53	Positive
6	27.67	27.35	Positive	28.97	27.34	Positive
7	33.11	32.1	Positive	34.73	31.75	Positive
8	29.25	28.62	Positive	30.57	29.21	Positive
9	29.59	28.41	Positive	31.52	29.13	Positive
10	30.16	29.55	Positive	30.53	28.78	Positive
11	29.35	28.6	Positive	30.75	28.82	Positive
12	19.56	18.73	Positive	20.25	19.94	Positive
13	19.71	18.84	Positive	19.62	19.74	Positive
14	19.66	18.79	Positive	19.92	20.02	Positive
15	18.99	18.42	Positive	20.15	19.7	Positive
16	19.52	18.77	Positive	19.48	19.54	Positive
17	19.61	18.85	Positive	20.18	19.69	Positive
18	28.8	28.15	Positive	30.4	27.95	Positive
19	28.71	27.99	Positive	30.01	28.35	Positive
20	28.65	28.17	Positive	30.4	28.02	Positive
21	28.95	28.09	Positive	30.11	28.39	Positive
22	28.51	27.71	Positive	30.53	27.89	Positive
23	28.86	27.68	Positive	30.11	27.99	Positive
24	30.72	30.11	Positive	33.72	31.13	Positive
25	31.12	30.43	Positive	33.33	30.09	Positive
26	30.37	29.79	Positive	33.16	30.29	Positive
27	29.98	29.27	Positive	31.31	29.65	Positive
28	31.35	30.08	Positive	30.93	29.66	Positive
29	23.68	22.82	Positive	24.52	23.58	Positive
30	23.49	22.83	Positive	24.61	24.57	Positive
31	23.02	22.39	Positive	24.44	23.96	Positive
32	23.5	22.73	Positive	24.66	24.32	Positive
33	23.27	22.71	Positive	24.46	24.71	Positive
34	22.81	22.51	Positive	24.32	24.25	Positive
35	33.2	32.6	Positive	33.9	33.6	Positive
36	29.51	27.91	Positive	29.19	28.95	Positive
37	26.18	25.87	Positive	26.38	26.46	Positive
38	29.27	27.66	Positive	27.41	27.34	Positive
39	27.75	28.66	Positive	28.53	28.53	Positive
40	25.1	26.4	Positive	27.4	27.1	Positive
41–125	Not detected	Not detected	Negative	Not detected	Not detected	Negative

Negative: Neither *E* nor *RdRp* genes were detected by the Taiwan CDC assay or neither *E* nor *ORF1ab* genes were detected by the LabTurbo AIO 48 platform.
* Positive: Both *E* and *RdRp* genes were detected by the Taiwan CDC assay or both *E* and *ORF1ab* genes were detected by the LabTurbo AIO 48 platform.

Table 5
Positive and negative agreement of LabTurbo AIO 48 SARS-CoV-2 assay versus Taiwan Centers for Disease Control (CDC) SARS-CoV-2 assay.

		Taiwan CDC assay		LabTurbo AIO 48 platform	
		E	*RdRp*	*E*	*ORF1ab*
Total Positive (n)		40		40	
Ct Value (n)	Low (>30)	8	8	6	6
	Medium (20–30)	25	25	27	30
	High (<20)	7	7	7	4
Total Negative (n)		85		85	

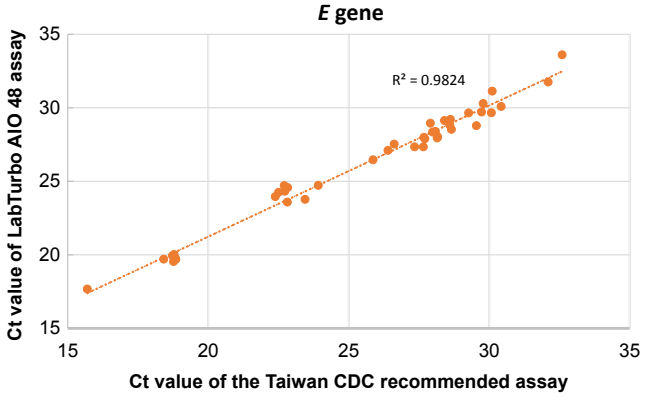

Fig. 1. Correlation of Ct values of clinical positive samples, using the Taiwan Centers for Disease Control (CDC) recommended assay and the LabTurbo AIO 48 assay, targeting the *E* viral gene for SARS-CoV-2 detection.

analysis, Investigation. **Chih-Kai Chang:** Formal analysis, Investigation. **Jung-Chung Lin:** Conceptualization, Methodology, Supervision. **Kuo-Ming Yeh:** Conceptualization, Methodology, Supervision. **Sheng-Kang Chiu:** Conceptualization, Methodology, Supervision. **Yi-Hui Wang:** Formal analysis, Investigation. **Shu-Jung Liao:** Formal analysis, Investigation. **Shih-Yi Li:** Formal analysis, Investigation. **Shan-Shan Hsieh:** Formal analysis, Investigation. **Cherng-Lih Perng:** Conceptualization, Methodology, Supervision. **Feng-Yee Chang:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Hung-Sheng Shang:** Conceptualization, Methodology, Supervision, Writing - review

& editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.12.003.

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