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Short communication

Analytical sensitivity comparison of 14 conventional and three rapid RT-PCR assays for SARS-CoV-2 detection

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ABSTRACT

Recent reports have compared the analytical sensitivities of some SARS-CoV-2 RT-PCR assays, but differences in the viral materials used for these evaluations made comprehensive conclusions difficult. We carried out a direct comparison of the analytical sensitivities of 14 conventional and three rapid RT-PCR assays for the detection of SARS-CoV-2. The comparison was performed utilizing a certified reference material for SARS-CoV-2 RNA that was serially two-fold diluted in RNA storage solution. Our results show that the analytical sensitivities of the 17 assays varied within an 8-fold range (100–800 copies/mL). Moreover, a trend with some rapid assays yielding slightly higher analytical sensitivities (2- to 4-fold) compared with conventional assays was observed. We conclude that most of the RT-PCR assays can be used for routine COVID-19 diagnosis, but some assays with the poorest analytical sensitivities may lead to false-negative results when used to identify asymptomatic individuals who can carry a low viral load but still be infectious. These findings should be kept in mind when selecting high-sensitivity and rapid assays.

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has significantly impacted both the healthcare system and economic activity. The standard method used for the early diagnosis and active monitoring of individuals potentially exposed to SARS-CoV-2 is a real-time reverse transcription polymerase chain reaction (RT-PCR) assay. Although the RT-PCR assay is highly sensitive, false-negative results do often occur because of various factors, including specimen type and quality, operating personnel, or test kit quality (Woloshin et al., 2020). Analytical sensitivity is an undisputedly crucial issue for accurate COVID-19 diagnosis (Wang et al., 2020). Currently, numerous SARS-CoV-2 RT-PCR diagnostic assays with varying claimed analytical sensitivities are being commonly used worldwide. There are several reports comparing analytical sensitivities between and among assays (Matsumura et al., 2021; Mostafa et al., 2020; Price et al., 2021; Smith et al., 2020; van Kasteren et al., 2020; Wang et al., 2020). However, these data were obtained by using different viral materials, such as synthetic RNA transcripts, extracted viral RNA, or clinical samples, which makes drawing comprehensive conclusions difficult. Here, we report a direct comparison among the analytical sensitivities (lower limit of detection [LoD]) of 17 widely available RT-PCR assays (14 conventional and three rapid detection assays), using certified reference material (CRM) for SARS-CoV-2 RNA.

Seventeen different commercial SARS-CoV-2 RT-PCR assays were selected, including 14 conventional assays with a reaction time of >1 h (Shanghai Liferiver Bio-tech Co., Ltd.; BGI Genomics Co., Ltd.; DAAN Gene Co., Ltd. of Sun Yat-sen University; Sansure Biotech Inc.; Shanghai BioGerm Medical Co., Ltd.; Beijing Applied Biological Technologies Co., Ltd. (XABT); Maccura Biotechnology Co., Ltd.; Wuhan Easy Diagnosis Biomedicine Co., Ltd.; Shanghai Fosun Long March Medical Science Co., Ltd.; Beijing Kinghawk Pharmaceutical Co., Ltd.; Jiangsu Bioperfectus Technologies Co., Ltd.; Beijing NaGene Diagnosis Reagent Co., Ltd.; Zhejiang Orient Gene Biotech Co., Ltd.; and Coyote Bioscience) (Table 1) and three rapid detection kits with a reaction time of <45 min (DAAN Gene Co., Ltd. of Sun Yat-sen University; Sansure Biotech Inc.; and Coyote Bioscience) (Table 2). All 17 included RT-PCR assays were approved by the China National Medical Products Administration (NMPA) and subsequently approved by the European Medicines Agency, US Food and Drugs Administration, and/or World Health Organization for use globally.

The CRM for SARS-CoV-2 RNA (National Institute of Metrology [NIM], code GBW(E) 091099, Beijing, China), prepared using purified genomic RNA of SARS-CoV-2 from positive samples from patients diagnosed with COVID-19, was purchased from NIM and used to

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Table 1
Characteristics and analytical sensitivity of 14 approved conventional RT-PCR assays.

Assay[a]	Target gene(s)	RNA extraction method	Volume of RNA template / Each PCR reaction (μL)	Positivity rate (no. of positive samples/total no.) (%) at diluted concentrations (copies/mL)[b,c,d]					Claimed LOD (copies/mL)	Thermal cycling condition	Reaction time (min.)	Regulatory status[f]
				800	400	200	100	50				
Liferiver	ORF1ab/N/E	Magnetic Bead method (Liferiver) / QIAamp Viral RNA Mini Kit (QIAGEN)	5/25	20/20 (100 %)	**19/20 (95 %)**	14/20 (70 %)	11/20 (55 %)	ND[e]	1000	10 min at 45 °C, 3 min at 95 °C, 45 cycles of 15 s at 95 °C and 30 s at 58 °C	82	NMPA, CE-IVD, WHO EUL
BGI	ORF1ab	TIANamp Virus RNA Kit (Tiangen) / QIAamp Viral RNA Mini Kit (QIAGEN)	10/30	20/20 (100 %)	**19/20 (95 %)**	16/20 (80 %)	12/20 (60 %)	ND	100	20 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 30 s at 60 °C	89	NMPA, CE-IVD, WHO EUL, FDA EUA
DAAN Gene	ORF1ab/N	Magnetic Bead method (DAAN Gene)	5/25	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	13/20 (65 %)	ND	500	15 min at 50 °C, 15 min at 95 °C, 45 cycles of 15 s at 94 °C and 45 s at 55 °C	110	NMPA, CE-IVD, WHO EUL
Sansure	ORF1ab/N	One-step method (Sansure) / Magnetic bead method (Sansure)	10/40	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	17/20 (85 %)	ND	200	30 min at 50 °C, 1 min at 95 °C, 45 cycles of 15 s at 95 °C and 30 s at 60 °C	98	NMPA, CE-IVD, FDA EUA
BioGerm	ORF1ab/N	Magnetic bead method (BioGerm)	5/25	**20/20 (100 %)**	17/20 (85 %)	10/20 (50 %)	7/20 (35 %)	ND	1000	10 min at 50 °C, 5 min at 95 °C, 40 cycles of 10 s at 95 °C and 40 s at 55 °C	81	NMPA, CE-IVD
XABT	ORF1ab/N	Magnetic bead method (XABT)	5/20	20/20 (100 %)	20/20 (100 %)	**19/20 (95 %)**	14/20 (70 %)	ND	200	10 min at 45 °C, 5 min at 95 °C, 45 cycles of 15 s at 95 °C and 45 s at 60 °C	92	NMPA, CE-IVD, WHO EUL
Maccura	ORF1ab/N/E	Magnetic Bead method (Maccura) / QIAamp Viral RNA Mini Kit (QIAGEN)	20/40	20/20 (100 %)	**20/20 (100 %)**	18/20 (90 %)	8/20 (40 %)	ND	1000	15 min at 55 °C, 2 min at 95 °C, 40 cycles of 15 s at 95 °C and 35 s at 58 °C	80	NMPA, CE-IVD, FDA EUA
Mdeasydiagnosis	ORF1ab/N	Magnetic Bead method (Mdeasydiagnosis) / QIAamp Viral RNA Mini Kit (QIAGEN)	5/25	**20/20 (100 %)**	18/20 (90 %)	10/20 (50 %)	6/20 (30 %)	ND	500	15 min at 50 °C, 30 s at 95 °C, 40 cycles of 3 s at 95 °C and 40 s at 60 °C	72	NMPA, CE-IVD, FDA EUA
Fosun-diagnostics	ORF1ab/N/E	Magnetic Bead method (Fosun-diagnostics / Genolution) / QIAamp Viral RNA Mini Kit (QIAGEN)	10/30	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	12/20 (60 %)	ND	300	15 min at 50 °C, 3 min at 95 °C, 5 cycles of 5 s at 95 °C and 40 s at 60 °C, 40 cycles of 5 s at 95 °C and 40 s at 60 °C	83	NMPA, CE-IVD, WHO EUL, FDA EUA
Kinghawk	ORF1ab/N	Magnetic Bead method (Kinghawk) / QIAamp Viral RNA Mini Kit (QIAGEN)	5/25	20/20 (100 %)	**20/20 (100 %)**	17/20 (85 %)	13/20 (65 %)	ND	500	30 min at 50 °C, 3 min at 95 °C, 40 cycles of 10 s at 95 °C and 30 s at 55 °C	93	NMPA, CE-IVD
Bioperfectus	ORF1ab/N	Magnetic Bead method (Bioperfectus) /	5/25	**20/20**	18/20	12/20	4/20 (20 %)	ND	350	10 min at 50 °C, 1 min at 97 °C, 45	74	NMPA, CE-IVD, WHO

(*continued on next page*)

Table 1 (*continued*)

Assay[a]	Target gene(s)	RNA extraction method	Volume of RNA template / Each PCR reaction (μL)	Positivity rate (no. of positive samples/total no.) (%) at diluted concentrations (copies/mL)[b,c,d]					Claimed LOD (copies/mL)	Thermal cycling condition	Reaction time (min.)	Regulatory status[f]
				800	400	200	100	50				
		QIAamp Viral RNA Mini Kit (QIAGEN)		**(100 %)**	(90 %)	(60 %)				cycles of 5 s at 97 °C and 30 s at 58 °C		EUL, FDA EUA
NaGene	ORF1ab/N	One-step method (NaGene)	10/45	20/20 (100 %)	**20/20 (100 %)**	15/20 (75 %)	10/20 (50 %)	ND	200	15 min at 50 °C, 3 min at 94 °C, 42 cycles of 10 s at 94 °C and 35 s at 58 °C	80	NMPA, CE-IVD
Orientgene	ORF1ab/N	One-step method (Orientgene)	15/50	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	11/20 (55 %)	ND	1000	20 min at 50 °C, 90 s at 95 °C, 45 cycles of 5 s at 95 °C and 15 s at 58 °C	70	NMPA, CE-IVD
Coyotebio	ORF1ab/N	One-step method (Coyotebio)	15/50	20/20 (100 %)	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	6/20 (30 %)	400	5 min at 42 °C, 15 cycles of 10 s at 95 °C and 15 s at 50 °C, 1 min at 95 °C, 30 cycles of 10 s at 95 °C and 30 s at 55 °C	71	NMPA, CE-IVD

[a] The names of the 14 approved conventional assays are all "Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)". The web links for the 14 approved conventional assays are Liferiver, http://www.liferiverbiotech.com/; BGI, https://www.bgi.com/us/; DAAN Gene, http://en.daangene.com/; Sansure, http://eng.sansure.com.cn/; BioGerm, http://bio-germ.com/; XABT, http://www.x-abt.com/; Maccura, https://www.maccura.com/; Mdeasydiagnosis, http://www.mdeasydiagnosis.com/; Fosun-diagnostics, http://www.lm-diagnostics.com.cn/; Kinghawk, http://www.kinghawk828.com/; Bioperfectus, http://www.s-sbio.com/; NaGene, http://www.nagened.com/; Orientgene, http://www.orientgene.com/; and Coyotebio, http://www.coyotebio.com/.

[b] Copies/mL determined with ddPCR by National Institute of Metrology (NIM, China) for the ORF1ab target gene.

[c] The limit of detection (LoD) by positivity rate for each assay is highlighted in bold.

[d] All assays were run on an ABI 7500 thermocycler (Thermo Fisher Scientific).

[e] ND, not done.

[f] NMPA: National Medical Products Administration; CE-IVD: Conformité Européenne-In Vitro Diagnostics; WHO EUL: World Health Organization Emergency Use Listing; FDA EUA: US Food and Drug Administration Emergency Use Authorization.

evaluate and compare the LoDs of the 17 assays. The concentrations of ORF1ab, N, and E gene in the CRM were determined with droplet digital PCR as 6.89×10^5, 1.36×10^6, and 8.04×10^5 copies/mL, respectively. The CRM was stored at −80 °C and subjected to only one freeze-thaw cycle. The LoD was determined by using CRM with two-fold serial dilutions of the ORF1ab gene to obtain the following five concentrations: 800, 400, 200, 100, and 50 copies/mL. Dilutions were prepared using Ambion RNA storage solution (Thermo Fisher Scientific) to prevent potential RNA degradation and were tested within 48 h (2–8 °C) to allow an unbiased comparison among the assays. Twenty replicates of each sample were tested per concentration. All 14 conventional real-time RT-PCR assays were performed on an ABI 7500 thermocycler (Thermo Fisher Scientific). The three rapid detection assays each required designated thermocyclers: iPonatic, Flash20, and AGS8830-8 were used for the Sansure, Coyotebio, and DAAN Gene assay, respectively. Testing and results interpretation were performed separately in accordance with each manufacturer's instructions. The LoD was defined as the lowest concentration at which 95 % of the tested replicates were detected.

Applying the above evaluation criteria, we found that the LoD of different assays varied within an 8-fold range among the 14 conventional assays, with the Coyotebio assay having the lowest LoD at 100 copies/mL and the BioGerm, Mdeasydiagnosis, and Bioperfectus assays having the highest LoD at 800 copies/mL (Table 1). The difference in analytical sensitivity between assays is probably not related solely to the volume of input RNA template/PCR reaction but is likely also a result of technical deficiencies (unreasonable primer design, primer or probe impurities, and reagent instability) or batch-to-batch variance (Wang et al., 2020). Some assays containing multiple viral targets (two or three) showed lower or similar sensitivities compared with the BGI assay, which has only one PCR target, indicating that using only a single target is sufficiently sensitive for SARS-CoV-2 detection (Price et al., 2021). Additionally, our results demonstrate that 28.6 % (4/14) of the tested assays could not meet their claimed sensitivity. Considering that differences in RNA extraction methods could further widen the gap in LoDs, there may be more real LoDs that fall outside the scope of the claimed LoDs.

To meet the urgent need for screening and diagnosis of SARS-CoV-2-infected individuals, three rapid RT-PCR tests with different reaction times and throughput capacities have been approved by NMPA and are widely used in clinical practice in China (Table 2). The Sansure rapid assay can run only a single specimen at a time, with results available in ≤45 min. The rapid Coyotebio and DAAN Gene assays each have higher throughputs of up to 4 and 8 samples per run, with results available in 29 and 35 min, respectively. Our analysis revealed that the LoDs of the Sansure, Coyotebio, and DAAN Gene assays were 400, 100, and 800 copies/mL, respectively. There was a trend with the Sansure and DAAN Gene rapid assays yielding slightly higher LoDs (2- to 4-fold) compared with conventional assays, but the Coyotebio assay showed the same high sensitivity as conventional assays. Thus, our results further confirm that some rapid assays can also reliably detect low-viral-concentration specimens (Moran et al., 2020).

Our study has limitations to consider. We used different RT-PCR assays within a specific batch for the direct comparison of their LoDs. However, batch-to-batch variability exists among tests by the same

Table 2
Characteristics and analytical sensitivity of three approved rapid detection RT-PCR Assays.

Assay[a]	Target gene(s)	RNA extraction method	Volume of RNA template / Each PCR reaction (μL)	Positivity rate (no. of positive samples/total no.) (%) at diluted concentrations (copies/mL)[b,c,d]					Claimed LOD (copies/mL)	Thermal cycling condition	Reaction time (min.)	Number of samples tested per run	Regulatory status[f]
				800	400	200	100	50					
Sansure	ORF1ab/N	One-step method (Sansure)	5/20.5	20/20 (100 %)	**20/20 (100 %)**	17/20 (85 %)	15/20 (75 %)	ND[e]	200	10 min at 50 °C, 1 min at 95 °C, 45 cycles of 1 s at 95 °C and 20 s at 60 °C	42	1	NMPA, CE-IVD
Coyotebio	ORF1ab/N	One-step method (Coyotebio)	15/52	20/20 (100 %)	20/20 (100 %)	20/20 (100 %)	**20/20 (100 %)**	10/20 (50 %)	400	3 min at 42 °C, 15 cycles of 3 s at 96 °C and 5 s at 55 °C, 30 cycles of 3 s at 96 °C and 10 s at 55 °C	29	4	NMPA, CE-IVD
DAAN Gene	ORF1ab/N	One-step method (DAAN Gene)	5/25	**20/20 (100 %)**	18/20 (90 %)	10/20 (50 %)	6/20 (30 %)	ND	500	2 min at 50 °C, 2 min at 95 °C, 10 cycles of 5 s at 95 °C and 10 s at 60 °C, 32 cycles of 5 s at 95 °C and 10 s at 60 °C	35	8	NMPA, CE-IVD

[a] The names of the three approved rapid assays are all "Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)". The web links for the three approved rapid detection assays are Sansure, http://eng.sansure.com.cn/; Coyotebio, http://www.coyotebio.com/; and DAAN Gene, http://en.daangene.com/.
[b] Copies/mL determined with ddPCR by National Institute of Metrology (NIM, China) for the ORF1ab target gene.
[c] The limit of detection (LoD) by positivity rate for each assay is highlighted in bold.
[d] Three rapid detection assays require designated thermocycler with iPonatic for Sansure, Flash20 for Coyotebio and AGS8830−8 for DAAN Gene, respectively.
[e] ND, not done.
[f] NMPA: National Medical Products Administration; CE-IVD: Conformité Européenne-In Vitro Diagnostics.

manufacturer (Lu et al., 2020), which means that not all of our results can reflect the true analytical sensitivity of the tested assays. Furthermore, because we performed our comparison using a certified genomic RNA reference material, our experiments did not evaluate the nucleic acid extraction step. There is a true need for developing a more suitable reference material comprising a whole virus preparation to evaluate the LoDs for the entire process of various assays.

This is one of the first studies to use CRM for making a comprehensive and independent comparison among the analytical sensitivities of SARS-CoV-2 RT-PCR assays. Our findings show that the analytical sensitivities differ within an 8-fold range (100–800 copies/mL) among conventional and rapid assays, indicating that most of them can be used for routine COVID-19 diagnosis. However, some assays with the poorest analytical sensitivities may produce false-negative results when used to identify asymptomatic individuals who can carry a low viral load but still be infectious. Notably, the measured LoDs obtained using the CRM could help with the selection of appropriate assays. Furthermore, we advise each laboratory to locally validate analytical sensitivities and check batch-to-batch differences when selecting and using a SARS-CoV-2 RT-PCR assay; doing so could provide more accurate testing results and help better control the ongoing COVID-19 pandemic. More importantly, considering the possible inaccurate results arising from using RT-PCR with inadequate detection sensitivity, our data suggest that multiple detection methods, including antibody test, should be used in complement to improve the diagnostic effect, especially for the diagnosis of suspected COVID-19 patients with negative RT-PCR results and for the identification of asymptomatic infections.

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CRediT authorship contribution statement

Xueliang Wang: Conceptualization, Investigation, Methodology, Writing - original draft. **Zhongqiang Huang:** Data curation, Methodology, Validation. **Jian Song:** Data curation, Investigation, Validation. **Yanqun Xiao:** Resources, Formal analysis, Writing - review & editing. **Hualiang Wang:** Data curation, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Lu, Y., Li, L., Ren, S., Liu, X., Zhang, L., Li, W., Yu, H., 2020. Comparison of the diagnostic efficacy between two PCR test kits for SARS-CoV-2 nucleic acid detection. J. Clin. Lab. Anal. 34, e23554.
Matsumura, Y., Shimizu, T., Noguchi, T., Nakano, S., Yamamoto, M., Nagao, M., 2021. Comparison of 12 molecular detection assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J. Mol. Diagn. 23, 164–170.
Moran, A., Beavis, K.G., Matushek, S.M., Ciaglia, C., Francois, N., Tesic, V., Love, N., 2020. Detection of SARS-CoV-2 by use of the cepheid xpert xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 assays. J. Clin. Microbiol. 58, e00772.
Mostafa, H.H., Hardick, J., Morehead, E., Miller, J.A., Gaydos, C.A., Manabe, Y.C., 2020. Comparison of the analytical sensitivity of seven commonly used commercial SARS-CoV-2 automated molecular assays. J. Clin. Virol. 130, 104578.

Price, T.K., Bowland, B.C., Chandrasekaran, S., Garner, O.B., Yang, S., 2021. Performance characteristics of severe acute respiratory syndrome coronavirus 2 PCR tests in a single health system: analysis of &10,000 results from three different assays. J. Mol. Diagn. 23, 159–163.

Smith, E., Zhen, W., Manji, R., Schron, D., Duong, S., Berry, G.J., 2020. Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. J. Clin. Microbiol. 58, e01134.

van Kasteren, P.B., van der Veer, B., van den Brink, S., Wijsman, L., de Jonge, J., van den Brandt, A., Molenkamp, R., Reusken, C., Meijer, A., 2020. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J. Clin. Virol. 128, 104412.

Wang, X., Yao, H., Xu, X., Zhang, P., Zhang, M., Shao, J., Xiao, Y., Wang, H., 2020. Limits of detection of six approved RT-PCR kits for the novel SARS-coronavirus-2 (SARS-CoV-2). Clin. Chem. 66, 977–979.

Woloshin, S., Patel, N., Kesselheim, A.S., 2020. False negative tests for SARS-CoV-2 infection - challenges and implications. N. Engl. J. Med. 383, e38.