Limits of Detection of Six Approved RT–PCR Kits for the Novel SARS-coronavirus-2 (SARS-CoV-2)

Running Title: LoDs of six SARS-CoV-2 RT–PCR Kits

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To the Editor,

The novel SARS coronavirus 2 (SARS-CoV-2, previously 2019-nCoV) can cause lethal coronavirus disease 2019 (COVID-19) (1). Since its outbreak in December 2019, COVID-19 has posed a great threat to human health and life in China and the world (2). Nucleic acid testing is the gold standard method for confirming infection (3). Many real-time reverse transcription (RT)–PCR kits have been developed and used by the World Health Organization, the United States Centers for Disease Control and Prevention, the Chinese Center for Disease Control and Prevention, and private companies. However, in China, false-negative results have been reported at rates as high as 20 to 40 percent in cases for which both clinical symptoms and imaging evidence raised strong suspicions of disease (4). False negatives may be caused by various factors, including the specimen source, timing of sampling, personnel operation, and the test kit quality.

The limit of detection (LoD), the lowest analyte concentration that a kit can detect, is an important performance parameter in evaluating kit quality. To cope with the COVID-19 epidemic, the China National Medical Products Administration (NMPA) approved six RT–PCR kits for SARS-CoV-2, and some of which subsequently received CE marking. However, because the research and development time was short, the approved kits were not verified or optimized with appropriate numbers of clinical samples, which may have affected their LoDs. To examine whether LoD is a factor contributing to the observed false-negative results, we evaluated and compared the LoDs of these six kits using real viral RNA.

Viral RNA was extracted from cultured SARS-CoV-2 (SARS-CoV-2/ZJU-01/Human/2020) with the QIAamp Viral RNA Mini Kit (Qiagen). To verify its applicability, the viral RNA was tested with the six kits provided by Shanghai Liferiver Bio-tech Co., Ltd, Wuhan Huada Bio-tech Co., Ltd, Shanghai GeneoDx Biotech Co., Ltd, DAAN Gene Co., Ltd of Sun Yat-sen University, Sansure Biotech Inc., and Shanghai BioGerm Medical Co., Ltd. The different target genes (**Table 1**) produced typical S-shaped amplification curves, indicating that the RNA could be used in the six kits to evaluate their LoDs.

The viral RNA concentration was determined with RT–droplet digital PCR (RT–ddPCR), which allows the absolute quantification of viral RNA by counting single molecules, without reference to an external standard curve. The RT–ddPCR primers and probes were designed to target ORF1ab,

N gene, and E gene (5). ORF1ab-F: CCCTGTGGGTTTTACACTTAA, ORF1ab-R: ACGATTGTGCATCAGCTGA, ORF1ab-P: FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1; N-F: GGGGAACTTCTCCTGCTAGAAT, N-R: CAGACATTTTGCTCTCAAGCTG, N-P: FAM-TTGCTGCTGCTTGACAGATT-TAMRA; E-F: ACAGGTACGTTAATAGTTAATAGCGT, E-R: ATATTGCAGCAGTACGCACACA, N-P: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ. The 20 μL reaction mixture contained 5 μL of One-Step RT–ddPCR Supermix (Bio-Rad), 2 μL of One-Step RT–ddPCR reverse transcriptase (Bio-Rad), 1 μL of 300 mmol/L DTT (Bio-Rad), 1 μL of mixed primers and probe (600 nmol/L primers and 200 nmol/L probe), 5 μL of 7.6 ng/μL RNA template, and 6 μL of RNase-free water. Each reaction mix was analyzed with the QX ONE Droplet Digital PCR (ddPCR) System (Bio-Rad). The thermal cycling conditions were: 45 °C for 10 min (reverse transcription); 95 °C for 5 min; and 40 cycles of 95 °C for 15 s and 58 °C for 30 s. The average concentrations of ORF1ab, N gene, and E gene were 4.16×10^5, 5.33×10^5, and 5.04×10^5 copies/mL respectively, so the average concentration of viral RNA was 4.84×10^5 copies/mL.

The serially two-fold diluted viral RNA was detected 20 times for each concentration. Following guidelines in the Clinical Laboratory Standards Institute (CLSI) document EP17-A, the lowest concentration level with a detection rate of 95% for positive results was taken as the LoD for each kit. The LoDs of four of the kits were 484 copies/mL, whereas the LoD of BioGerm was 968 copies/mL and the LoD of GeneoDx was only 7744 copies/mL, giving a maximum 16-fold difference (**Table 1**). The poor LoD of the latter may be attributable to technical deficiencies in the product's manufacture, including unreasonable primer design, primer or probe impurities, reagent instability, or inappropriate reagent ratios. The low sensitivity of the kit implies that it may fail to identify many COVID-19 patients who consequently would be unlikely to receive appropriate treatment in time, hindering the prevention and control of the epidemic.

Our results show that the LoDs of the six commercial kits approved by NMPA differ substantially, with the poorest LODs likely leading to false-negative results when RT–PCR is used to detect SARS-CoV-2 infection. Manufacturers should analyze the existing problems according to the clinical application and further improve their products. Laboratories should verify and

compare the performances between kits from different manufacturers and different batches before their routine use. Such measures should reduce the clinical risks associated with false-negative results and more effectively control the spread of COVID-19 throughout the world.

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Table 1 Characteristics and limits of detection of six approved SARS-CoV-2 RT–PCR kits

Kits [a,b]	Target genes	RNA template volume (μl)	Each PCR reaction volume (μl)	SARS-CoV-2 RNA with different concentrations (copies/mL) [c]					
				7744	3872	1936	968	484	242
Liferiver	ORF1ab/N/E	5	25	100%	100%	100%	100%	100%	90%
Huada	ORF1ab	10	30	100%	100%	100%	100%	100%	90%
GeneoDx	ORF1ab/N	2	20	100%	70%	50%	35%	/	/
DAAN	ORF1ab/N	5	25	100%	100%	100%	100%	100%	35%
Sansure	ORF1ab/N	10	40	100%	100%	100%	100%	100%	90%
BioGerm	ORF1ab/N	5	25	100%	100%	100%	100%	80%	35%

[a] The six commercial kits have been approved by NMPA, and four have received CE marking (Liferiver, Huada, DAAN, and Sansure).

[b] The web links for the six approved kits are Liferiver, http://www.liferiverbiotech.com/; Huada, https://www.bgi.com/us/; GeneoDx, http://www.geneodx.com/; DAAN, http://en.daangene.com/; Sansure, http://eng.sansure.com.cn/; and BioGerm, http://bio-germ.com/.

[c] All kits detected the viral RNA on the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific).