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Short communication

## A localized small-scale external quality assessment (EQA) for PCR testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the molecular laboratories

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## ABSTRACT

The aim of this study was to estimate the PCR results for SARS-CoV-2 testing in 32 participating laboratories in a localized small-scale external quality assessment (EQA) scheme. EQA samples were distributed to the participants and detected immediately on the day of delivery. Qualitative results were submitted to the EQA provider, including negative or positive results along with cycle threshold (Ct) values for different target genes. Although the variability of Ct values differed among the laboratories in the EQA, a total of 32 (100 %) participants reported correct qualitative results. The study showed that the mean loads of *N* or *E* gene were higher than those of *ORF1ab* in SARS-CoV-2 RNA samples. Regardless of the analyzed gene target, the mean Ct values for weak positive and positive samples varied by fewer than 1.74 and 1.91 cycles, respectively. Less than 12 % of reported Ct values for *ORF1ab* and *N* genes deviated by more than  $\pm 4$  cycles (maximum:  $-9.92$  cycles), while none deviated by more than  $\pm 4$  cycles for the *E* gene. The current EQA program can provide a robust practical basis for follow-up planning to conduct evaluations for SARS-CoV-2 PCR testing and other novel emerging pathogens in the future.

## 1. Introduction

Robust, accurate, and reproducible molecular testing is essential to control the novel coronavirus disease (COVID-19), needing the cooperation of public health agencies, clinical laboratories, and industry (Binnicker, 2020). Viral nucleic acid testing has played an important role in control of the new disease, being regarded as the diagnostic gold standard. Many commercial kits detecting SARS-CoV-2 continue to come into the market, and laboratory workers must perform verification testing before carrying out testing of clinical samples. Quality management and improvement within the laboratories require urgent assistance from external quality assessment (EQA), especially for newly established molecular laboratories. Testing of standardized EQA samples could assess the laboratories' abilities and identify inaccurate or suboptimal combinations of reagents and instruments that may be used by some laboratories. Moreover, false-positive and false-negative results should be analyzed thoroughly. This approach could offer valuable practical suggestions to some EQA panel members for increasing assay reliability and improving their capabilities.

## 2. Materials and methods

Five samples (500  $\mu$ L each) provided to each enrolled laboratory in the EQA program included one SARS-CoV-2-negative sample (containing mixed MERS and SARS [ $1 \times 10^4$  copies/mL, each]), one SARS-CoV-2-negative sample (negative sample from healthy individual), and three random samples ranging from weak positive ( $1.61 \times 10^3$  copies/mL) to positive ( $1.45 \times 10^4$  copies/mL). All samples were customized: virus-like particles (VLPs) were diluted with phosphate buffered saline at suitable dilution ratios, then were subsequently used for RNA extraction and amplification. cDNA copies were quantified by a digital PCR system for deducing the initial VLP concentration.

All EQA samples were distributed to each participating molecular laboratory via dry ice delivery on the scheduled day. We recommended that the EQA samples be handled according to the laboratory biosafety interim guidance related to SARS-CoV-2 issued by the World Health Organization (Organization WH, 2020). Recommended volumes for RNA extraction and input for PCR testing were 200  $\mu$ L and 5  $\mu$ L, respectively. Requested EQA data included RNA extraction/amplification platforms, routine PCR reagents, negative or positive

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results, and raw Ct values for different gene targets (*ORF1ab*, *N*, and *E* gene), which were submitted online to the EQA provider on the date of sample delivery. Each laboratory could retest the remaining EQA sample volume (stored at  $-20^{\circ}\text{C}$ ) on the same day using alternative platforms and reagents, but those data were not required to be returned in this EQA program.

### 3. Results

As shown in Table 1, 32 laboratories were enrolled in the EQA program, utilizing diverse test systems and reagents comprising 18 different extraction instruments, eight different amplification instruments, and nine different PCR amplification reagents. In this study, all participating laboratories detected SARS-CoV-2 by real-time PCR. According to the returned information, all enrolled laboratories followed the advised extraction and input volumes for analysis. All the EQA samples containing SARS-CoV-2 RNA were successfully detected for the tested target genes, while all SARS-CoV-2-negative EQA samples were correctly identified as negative.

As shown in Table 2 and Fig. 1, different number Ct values of weak positive and positive *ORF1ab/N/E* gene were returned in corresponding laboratories for the randomness of EQA specimen distribution. The mean Ct values of the *N* and *E* genes were lower than those obtained for the *ORF1ab* gene. Regardless of the gene target analyzed, the mean Ct values for the weak positive and positive samples varied by fewer than 1.74 and 1.91 cycles, respectively. More than 61.7 % and 47.1 % of Ct values for weak positive and positive samples, respectively, deviated from the corresponding mean values by  $< \pm 2$  cycles, although this varied between target genes (Table 2). Deviation by  $> \pm 4$  cycles from mean Ct values was low, with *ORF1ab* showing strongest deviation at 11.8 % in the positive sample group, while no deviation by  $> \pm 4$  cycles was found in the *E*- gene. Ct values for target genes could represent important components of laboratory potential risk control strategies,

**Table 1**

Diverse test system/reagent combinations from 32 laboratories enrolled in the EQA.

	18 different Extraction platforms	8 different Amplification platforms	9 different PCR amplification reagents
1	SHZJ (4); DA (2); JSBF (2); TMF (1); XATL (2); ZKB (1); Manual (1)	SHHS (13)	SHZJ;(5); DA (4); ABT (1); SHBG (2); NG (1)
2	RD (1); MR (1); JSBF (2); TWNT (1); CQZY (1);	RD (6)	ABT (1); MC (1); SHBG (3); DA (1)
3	HYC (1); GW (1)	YRSW (2)	MC (1); GW (1)
4	HZB (1)	HZB (1)	DA (1)
5	XATL (1)	XATL (1)	SHZJ (1)
6	XATL (3); HAMT (1), JSBF (1)	TMF (5)	MC (2); ABT (1); BJKH (1); DA (1)
7	JSBF (1); Allsheng (1); HLKY (1)	ABI (3)	SHBG (2); SHFX (1)
8	BND (1)	AY (1)	DA (1)

Data are presented as (n of labs using these platforms/reagents). SHZJ: Shanghai ZJ BIO-TECH CO., LTD; XATL: Xi'an Tianlong Technology Co., Ltd; DA: DA'AN GENE CO., LTD; SHHS: Shanghai Hongshi Medical Technology Co., Ltd; JSBF: Jiangsu Biopertectus Technologies Co., Ltd; YRSW: Suzhou YR BIO-TECH CO., LTD; SHBG: Shanghai BioGerm Medical Technology Co., Ltd; ABT: Beijing Applied Biological Technologies Co., Ltd; NG: Beijing NaGene Diagnosis Reagent Co., Ltd; ZKB: Nanjing ZhongkeBio Medical Co., Ltd; HYC: HYCREATE BIOTECH Co., Ltd; GW: Geneway Biotech Co., Ltd; MC: Maccura Biotechnology Co., Ltd; HZB: Hangzhou Bioer Technology Co. Ltd; HAMT: Hamilton Company; TMF: Thermo Fisher Scientific; BJKH: Beijing Kinghawk Pharmaceutical Co., Ltd; RD: Roche Diagnostics; MR: Mindray Technology Co. Ltd; TWNT: Taiwan Advanced Nanotech Co. Ltd; CQZY: Chongqing ZY Co. Ltd; Allsheng: Hangzhou Allsheng Instruments Co., Ltd; HLKY: Beijing HLKY Co. Ltd; ABI: Applied Biosystems Co. Ltd; SHFX: Shanghai FX Co. Ltd; BND: BND Technologies Co., Ltd; AY: Hangzhou AnYu Technologies Co., Ltd.

especially when Ct values deviate dramatically from the mean Ct value.

### 4. Discussion

SARS-CoV-2 PCR testing is an important element for reducing transmission of this pathogen. Molecular testing with maximum accuracy is essential for all PCR laboratories and EQA represents a necessary procedure for ensuring reliable PCR results across all molecular laboratories involved in clinical testing. Although not being obtained from inactivated positive samples of confirmed cases, the SARS-CoV-2 RNA loads of EQA positive samples in the current EQA were far lower than the mean viral loads of most infected individuals (Pan et al., 2020). The assessment panel was well-timed to support the newly established molecular laboratories carrying out SARS-CoV-2 PCR testing, because these facilities were not included in the early large-scale EQA activity organized by the National Center for Clinical Laboratories (Wang et al., 2021). The EQA assessment described here was conducted on the scheduled date, including logistics service, PCR testing, and results reporting.

On the basis of previous EQA data, we found that false-positive or false-negative results were a potential problem (Wang et al., 2021; Fischer et al., 2020; Sung et al., 2020; Buchta et al., 2020). However, the specificity and sensitivity were simultaneously 100 % for all the participating laboratories in the current EQA program; the main reasons for which perhaps included the issuing of updated related laboratory operation guidelines by authorities and strict standardized permission for laboratories performing SARS-CoV-2 PCR testing in the capital. Although no laboratory in this EQA reported incorrect results, it remains necessary to keep a close watch on the possibility of contamination derived from manufacturers (Mogling et al., 2020).

Standardization is essential for laboratories to report comparable Ct values, because of a call for diagnostic tests with viral loads in the reports (Service, 2020). Consistent with previously reported results, we found that the sensitivity of *ORF1ab* detection was lower than that of *N* or *E* genes in our EQA samples (Wang et al., 2021). The laboratories participating in this EQA used a variety of extraction platforms, amplification platforms, and PCR reagents. Thus, Ct values obtained in different laboratories via different methods are challenging to compare. In agreement with other EQA results, the mean Ct values for the positive samples varied by fewer than 1.91 cycles (Buchta et al., 2020). However,  $\geq 47.1$  % of tests deviated from the mean Ct value by  $< \pm 2$  cycles and  $\leq 11.8$  % deviated by  $> \pm 4$  cycles in this EQA. We also found that some laboratories returned positive results along with maximum Ct value deviation from the corresponding mean Ct value, eg *ORF1ab* (-6.91) and *N* (-9.92) from weak positive samples, and *ORF1ab* (6.94) and *N* (-7.14) from positive samples. These data could provide an important warning for the EQA provider to further monitor specific laboratories. Laboratories should take into consideration concerns related to equipment calibration, pipette calibration, *in vitro* diagnostic assays performance, and personnel training. Therefore, the Ct values of positive results should be important components of future EQA programs for PCR testing of SARS-CoV-2.

This study had several limitations. First, point-of-care testing platforms were not included in the current EQA program. Second, information regarding alternative platforms and reagents in each laboratory were not collected and analyzed in the current EQA program.

In conclusion, this study summarizes an EQA for SARS-CoV-2 PCR testing including 32 public health organization laboratories in Beijing. Some laboratories should be focused on in a follow-up EQA owing to their much higher or lower Ct values compared with mean Ct values for corresponding gene targets. Molecular laboratories should perform verification experiments before carrying out routine PCR tests and monitor the laboratory output by meticulous quality management. Local small-scale EQAs can be useful for molecular laboratories involved in SARS-CoV-2 PCR testing, particularly those with little previous experience, to increase the quality of results and identify potential weaknesses.

**Table 2**

Mean Ct values and deviation from the mean of positive results from laboratories enrolled in the EQA.

	Weak positive EQA samples			Positive EQA samples		
	<i>ORF1ab gene</i>	<i>N gene</i>	<i>E gene</i>	<i>ORF1ab gene</i>	<i>N gene</i>	<i>E gene</i>
The participant laboratories	31	31	10	30	30	9
The total amount of reported Ct values	60	60	17	34	34	11
The mean Ct value	32.35	31.3	30.61	31.43	29.91	29.52
< ±2	45/60 (75 %)	37/60 (61.7 %)	15/17 (88.2 %)	16/34 (47.1 %)	18/34 (52.9 %)	9/11 (81.8 %)
> ±4	3/60 (5%)	4/60 (6.7%)	0/17	4/34 (11.8 %)	3/34 (8.8 %)	0/11
Negative/positive maximum	-6.91, 5.27	-9.92, 5.28	-2.89, 2.46	-5.51, 6.94	-7.14, 5.35	-3.29, 1.58

Data are presented as (n) or n (%).

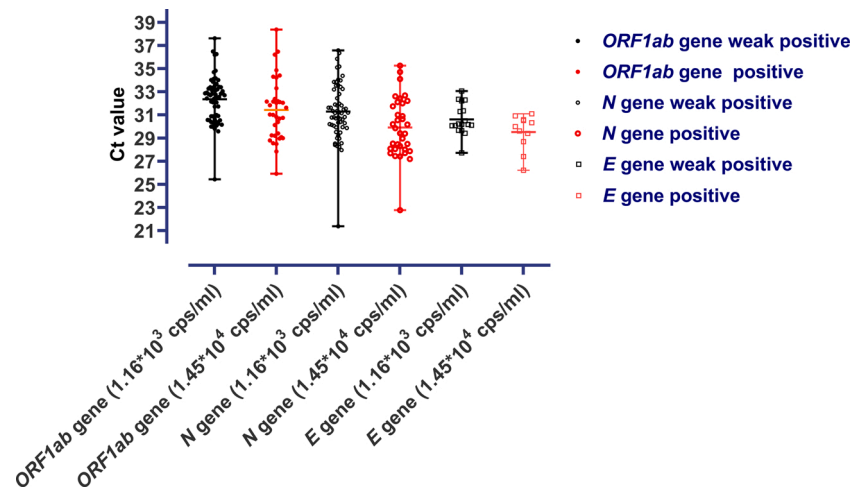


Fig. 1. Variability of Ct values for individual gene targets obtained from 32 laboratories in the EQA.

**Author statement**

**Rong-Hai Li (First Author):** Data curation, Visualization, Investigation.

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**Declaration of Competing Interest**

The authors report no declarations of interest.

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