Short Communication

THE APPLICATION OF AN IN-HOUSE REAL-TIME RT-PCR ASSAY AS A SCREENING TOOL FOR COVID-19 CASES DURING THE FIRST WAVE OF THE PANDEMIC IN MALAYSIA

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ABSTRACT

In December 2019, a global alert was warranted on the emergence of SARS-CoV-2, a novel coronavirus in China. The full genome of the virus was released within a few days. As part of the preparedness in facing the possibility of regional spread of the virus, we set up an in-house Real-Time RT-PCR assay for an early screening of the novel coronavirus (SARS-CoV-2). The single target assay was useful in detecting COVID-19 from the early batches of suspected cases that were sent to our laboratory. The assay had successfully detected the first three COVID-19 contact cases in Malaysia which could have been missed due to the unavailability of a standard detection method at that time.

KEYWORDS: COVID-19, Real-Time RT-PCR, First wave

INTRODUCTION

In late December 2019, a novel virus provisionally called 2019-nCoV and later renamed SARS-CoV-2, was linked to an outbreak at a wholesale seafood market in Wuhan. The disease caused by the virus was later officially named COVID-19 (1). In Malaysia, a total of 22 COVID -19 positive cases were recorded in the first wave of the pandemic which began on 24 January 2020, of which 12 were reported to have travelled to countries or regions with COVID-19 outbreaks (2). As part of the outbreak preparedness, the Virology Unit, Institute for Medical Research, Ministry of Health Malaysia stepped up to setup a molecular assay for the detection of the SARS-CoV-2 virus prior to the emergence of the first wave. The first SARS-CoV-2 full genome sequence from China which was publicly available in the GenBank (Wuhan-Hu-1, accession number MN908947) was used as the reference sequence in a cascade of bioinformatic analysis during the assay setup.

Primers and probe sequences were designed at the conserved region of the envelope gene (E gene) of SARS-CoV-2 and SARS-like CoV's that shared the highest similarity to the novel coronavirus. (Table 1). The primers and probe design process involved alignment of the E gene sequence of SARS-CoV-2 and SARS-like CoV's using CLUSTAL Omega software (https://www.ebi.ac.uk/Tools/msa/clustalo/) (3), after which the conserved region was identified. The sequence from this region was then inserted into the Primer 3 v.0.4.0 software (https://bioinfo.ut.ee/primer3-0.4.0/) to design multiple combinations of primers and probe sequences. These combinations were assessed for secondary structure and determination of properties such as melting temperature (Tm) and GC content using the OligoAnalyzer 1.0 tool. The primer pairs and probe that surpassed the in-silico quality assessment were submitted for synthesis by Integrated DNA Technologies, Inc. (IDT).

Table 1: Primers and probe sequences used in the first-line screening assay for SARS-CoV-2

Primer/Probe	Sequence (5'-3')	Target	Nucleotide position
Forward primer (WH nCoV-F)	GTACGAACTTATGTACTCATT	E gene	26235 -26255
Reverse primer (WH nCoV -R)	CAGTAAGGATGGCTAGTG		26333-26350
Taqman Probe (WH nCoV-P)	CAGGTACGTTAATAGTTAATA		26270-26290

All viral RNAs used in the development of the assay were extracted using QIAamp Viral RNA Mini kit (Qiagen, Germany) following the manufacturer's instruction. Real-Time RT-PCR was optimized on a CFX-96 Thermal Cycler (Biorad, USA) in a final reaction volume of 25 µL consisting of 12.5 µL of 2X Reaction mix, 5.5 µL of RNase-free water, 0.5 µL of each primer (10 µM), 0.5 µL of probe (10 µM), 0.5 µL of SuperScript™ III RT/Platinum™ Taq Mix and 5 µL of RNA extract. Cycling conditions included reverse transcription at 50 °C for 30 min, incubation at 95 °C for 2 min, and 45 cycles of 95 °C for 15 s and 54 °C for 30 s. Since the assay was meant for the detection of a novel virus, a specific assay control was unavailable at that time. Therefore, the SARS-CoV-1 virus isolate shared by University Malaya Medical Centre (UMMC) was utilized as a positive control. The virus belonged to the same family as the SARS-CoV-2 and shared about 80% nucleotide sequence similarity.

The assay displayed a positive amplification in the FAM channel (cut-off Ct <40) corresponding to the detection of the SARS-CoV-2 genome and specificity testing indicated no cross-reactivity with other respiratory viruses. The developed assay was then shared with the National Public Health Laboratory (MKAK, Sungai Buloh) which enabled the successful detection of the first three contact cases of COVID-19 in Malaysia on 24th January 2020. The results were later reconfirmed and validated with the RdRp assay protocol released by Berlin/ WHO (4). The three cases included a 66-year-old woman and her two grandchildren, who were among the eight close contacts with the first positive case reported in Singapore (5). Subsequently, the assay was also utilized to screen a total of 583 cases under suspicion of COVID-19 infection and classified as patient under investigation (PUI). From these, 22 cases (Table 2) were detected as positive by the developed assay with ct value ranging from 17.00-38.50 and yielded 100% concordant results with the Berlin/WHO protocol. This in a way aided the preliminary validation of the in-house assay. The amplification curves for the representative cases are shown in Figure 1. It is noteworthy that the mean ct value for detection of SARS-CoV-2 from sputum,

nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) were 24.48, 31.13 and 34.04 respectively, indicating that the viral shedding is higher in the sputum, concurring with other study findings (6,7).

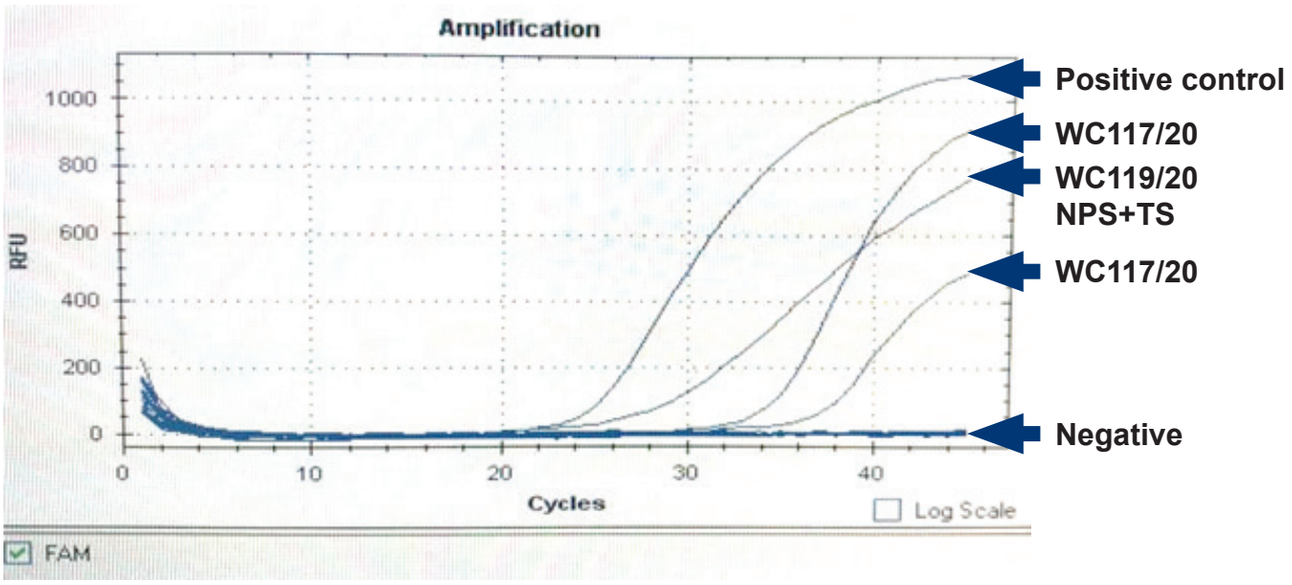

Figure 1. Amplification of SARS-CoV-2-19 by Taqman Real-Time-RT-PCR from representative samples. NPS: Nasopharyngeal swab; TS: Throat swab; OPS: Oropharyngeal swab

Table 2. Positive cases of SARS-CoV-2 detected by in-house Taqman Real-Time-RT-PCR

No	Lab ID	Type of specimen	Ct value
1	RM0202/20	Sputum	21.17
2	RM0206/20	NPS	27.73
3	RM0207/20	NPS	17.37
4	RM0208/20	NPS	35.30
5	WC117/20	NPS & OPS	33.36(NPS);36.69(OPS)
6	WC119/20	NPS+TS	28.48
7	WC230/20	NPS & OPS	32.01(NPS);36.68(OPS)
8	WC250/20	OPS & Sputum	30.79(OPS);27.79(Sputum)
9	WC313/20	OPS	30.25
10	WC336/20	OPS	32.35
11	WC355/30	NS+TS	36.69
12	WC407/30	NPS+OPS	32.05
13	WC408/20	NPS+OPS	35.78
14	WC413/20	OPS	21.79
15	WC440/20	NPS & OPS	26.65(NPS);35.43(OPS)
16	WC442/20	NS	37.00
17	WC487/20	NPS+OPS	35.93
18	WC465/20	OPS	37.45
19	WC627/20	OPS	25.65
20	WC702/20	NPS & OPS	36.07(NPS);38.57(OPS)
21	WC708/20	NPS	37.26
22	WC764/20	NPS & OPS	34.44(NPS);34.85(OPS)

A few strengths and limitations are inherent in this study. The assay was designed to target the E gene at the conserved region for SARS-CoV-1, SARS-like, Bat SARS-coronavirus and SARS-CoV-2, hence, it served as a first-line screening tool at the time no other diagnostic assay was available for COVID-19. Even though the developed assay was intended to target the SARS-like beta coronavirus family, however at the time of the outbreak there was no circulation of SARS or SARS-like viruses, therefore, it can be justified that the assay was useful for the specific detection of SARS-CoV-2. Importantly, the preparedness of a reference laboratory in facing a probable pandemic is apparent and the effort had helped the nation to accurately identify the first occurrence of the outbreak in the country [8]. Nonetheless, there was limited time to complete the validation of the assay particularly to test the limit of detection due to the immediate need to utilize the assay for a real-world diagnosis.

In conclusion, the availability of the genetic information of the SARS-CoV-2 within a short span of time ought to be applauded as this allowed scientist worldwide to develop their own molecular detection assay for COVID-19. In a similar vein, we have developed a Real-Time RT-PCR assay as a rapid diagnostic tool for the detection of SARS-CoV-2. The assay was found to be useful as an early screening tool for COVID-19 at the time of the unavailability of a standard detection assay.

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