Using the Azure Cielo™ Real-Time PCR System for SARS-CoV-2 viral RNA detection

Introduction

The outbreak of severe acute respiratory syndrome 2 (SARS-CoV-2) responsible for the pandemic of 2020 has taken the lives of 4.39M people to date[1]. To help contain the virus and reduce the number of fatalities, rapid and reliable diagnosis is critical.

The 'gold standard' for COVID-19 detection relies upon a well-established technique called reverse transcription quantitative polymerase chain reaction, or RT-qPCR. Briefly, this procedure involves RNA extraction, reverse transcription into complementary DNA or cDNA, and quantification of the transcript (specific to the viral genome) with a thermal cycler in real-time.

For early detection and diagnosis, it is imperative that real-time PCR instruments are capable of measuring very small amounts of viral RNA. Researchers estimate that the limit of detection (LOD), or the lowest concentration of RNA detected with > 95% confidence, can be as little as 1 copy of viral RNA per µL[2]. Therefore, in this technical note, we sought to determine the LOD for the Azure Cielo using SARS-CoV-2 RNA.

Materials and methods

The LOD, or analytical sensitivity, of the Azure Cielo Real-Time PCR instrument was tested using the FDA approved COVID-19 genesig® Real-Time PCR assay as described in the instructions for use (IFU). The positive control template (PCT) provided with the kit was used to generate a six-point standard curve as follows:

Dilution #	Dilution factor	Copies per µL
0	Stock	$1.25x10^5$
1	1/100	$1.25x10^3$
2	1/100	$1.25x10^1$
3	1/10	1.25
4	1/2	0.625
5	1/10	0.06

Table 1. PCT dilution series. Each dilution was measured in technical triplicate.

SARS-CoV-2 whole genomic RNA (Ref: 026N-03889) was obtained from the European Virus Archive-Global (EvaG) and a four-point serial dilution was generated as described in the IFU. Twenty replicates per dilution were measured.

The final composition of each reaction was:

- 8 µL of sample (PCT or SARS-CoV-2 RNA)
- 10 µL of oasig™ OneStep 2X RT-qPCR Master Mix
- 2 µL COVID-19 Primer & Probe

The reactions were loaded into a 96-well plate and the following amplification protocol was used:

1. reverse transcription at 55°C for 10 min
2. initial denaturation (Taq activation) at 95°C for 2 min
3. denaturation at 95°C for 10 sec
4. annealing and extension at 60°C for 60 sec, followed by a plate read
5. steps 3–4 were repeated 45X in total

Results and discussion

To correlate the number of copies of viral RNA/µL to Cq value, a standard curve from the PCT was generated using the dilution factors shown in Table 1 (Figure 1).

To determine the LOD, we compared the Cq values of SARS-CoV-2 RNA detected by the Cielo at dilution #'s 2–5 (Table 1). We found that at 12.5 copies/µL, 20/20 or 100% of the replicates were detected by the instrument and had a mean Cq of 30.49 (Figure 2, green). At 1.25 copies/µL, 20/20 (100%) of replicates were detected and had a mean Cq of 33.66 (Figure 2, yellow). At 0.625 copies/µL, 20/20 (100%) of the replicates were detected and had a Cq average of 34.80 (Figure 2, orange). At the final dilution of 0.06 copies/µL, 12/20 or 60% of the replicates were detected and the average Cq was 37.12 (Figure 2, red).

These results demonstrate that the LOD for the Cielo is 0.625 copies of SARS-CoV-2 RNA/µL, indicating that the Cielo can be used for rapid and reliable detection of COVID-19.

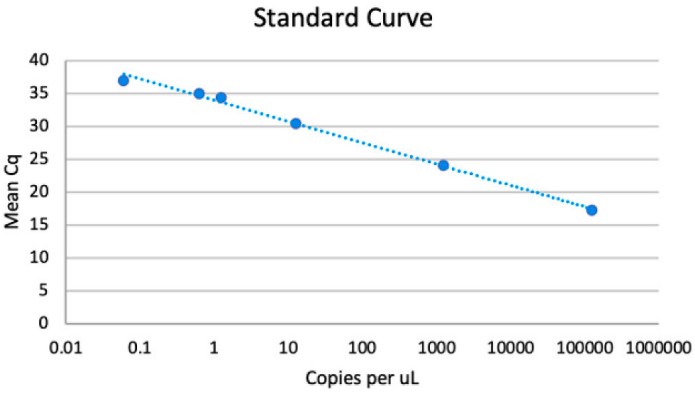

Figure 1. A six-point standard curve was used to estimate the number of copies of viral RNA/µL. The mean Cq value for each dilution is shown.

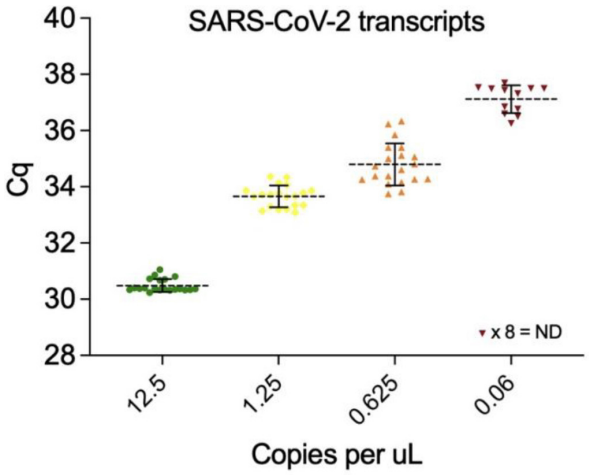

Figure 2. The number of copies of SARS-CoV-2 transcripts per µL as a function of Cq value. The dashed line represents the mean and error bars show the standard deviation (n= 20). At 0.06 copies/µL, 8 replicates were not detected (ND).

References

1. https://www.nytimes.com/interactive/2021/world/covid-cases.html
2. Arnaout R, Lee RA, Lee GR, Callahan C, Yen CF, Smith KP, Arora R, Kirby JE. SARS-CoV2 Testing: The Limit of Detection Matters. *bioRxiv* [Preprint]. 2020 Jun 4:2020.06.02.131144. doi: 10.1101/2020.06.02.131144.