

Gnomegen COVID-19 Real-Time Digital PCR Assay Kit: A Novel Solution for the Detection of the 19-nCoV Virus

HIGHLIGHTS:

- Highly sensitive detection of the 2019-nCoV Virus in comparison to rRT-PCR

INTRODUCTION:

Currently, using the existing qPCR technologies, the sensitivity of the rRT-PCR COVID-19 kits is not high and therefore false positives are prevalent [1]. This is seen particularly in documented cases in Wuhan. As epidemiologist Zhong Nanshan suggests, the most likely factor leading to repeated diagnoses was the current method of testing, which can lead to false positives [2]. Gnomegen seeks to combat the low sensitivity of current methods by using real-time digital PCR monitoring to increase the detection of the novel 2019-SARS-CoV-2.

Compared to qPCR, chip-based digital PCR uses microwell chips partitioned into 20,000 different microwells which allows for the absolute quantification of the target sequences. Unlike traditional qPCR, digital PCR provides a linear feedback to the number of copies present to allow for small fold change differences to be detected.

BENEFIT OF REAL TIME DIGITAL PCR SYSTEM:

Analytical validation data was initially generated using reference cDNA encoding N-gene for COVID-19. As shown in figure 1, results indicate that for the detection of the

novel 2019-SARS-CoV-2 virus, the limit of detection is 2.5 copies/rxn for digital PCR vs. 10 copies/rxn for qPCR. There is an increase from 62.5% detection rate to 95% detection rate when digital PCR is used.



Figure 1: Percent Positive Target Amplification Drt-PCR vs. qPCR. At a 95% positive amplification, result indicate, the limit of detection for Drt-PCR is 2.5 copies while the limit of detection for qPCR is 10 copies.

Currently in the market, methods for detecting COVID-19 are not sensitive enough for the detection of the novel SARS-CoV-2 virus. False negatives results are seen in the samples tested and thus will risk the current population by increasing the cases of unidentified patients harboring the 2019- SARS-CoV-2 virus. In response to the market, there is a need and a requirement for a more sensitive assay that would decrease the instances of false negatives. Gnomegen meets that need with Gnomegen COVID-19 RT Digital PCR Detection Kit.

CLINICAL PERFORMANCE:

In comparison to the qPCR system, the Gnomegen COVID-19 RT-Digital PCR Detection kit used with the digital PCR

system also demonstrated a higher sensitivity at a lower limit of detection when contrived clinical samples are used. The data below was obtained using contrived clinical matrices.

Table 1: LoD determination

Sample Type	Effective Concentration	Number of Samples	# of Positive Samples	%
Upper Respiratory Specimen	LoD (3 copies/reaction)	20	19/20 samples	95% Positive
	Negative	20	20/20 samples	0% Positive

Results are in accordance with cDNA reference standards. A limit of detection was determined to be 3 RNA copies / reaction.

When testing contrived clinical specimens using inactivated virus at the limit of detection for dPCR at 3 copies/reaction, the results indicate that of the 20 samples tested at negative and 20 samples tested at the LoD, only 75% of the samples tested positive using qPCR while 95% (19/20) samples tested positive using dPCR.

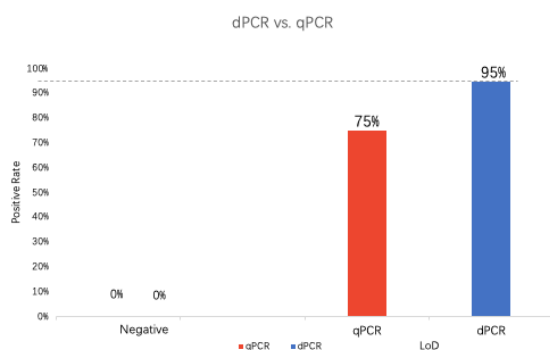


Figure 2: Percent Positive Target Amplification Drt-PCR vs. qPCR. Result indicate at the limit of detection for Drt-PCR at 3 copies using inactivated virus, 95% (19/20) samples were positive for Drt-PCR and 75% (15/20) samples were positive for qPCR.

Besides verifying LoD, a total of 120 contrived clinical matrices including both upper and lower respiratory samples were tested, a total of 40 negative samples, 60 samples at 1-2X LoD

and 20 samples at 3-5X LoD were tested. Of the samples tested, 40 negative samples are determined to be negative, indicating a 100% agreement with the expectation. Of the 80 positive samples tested, 78 samples are determined to be positive, indicating a 97.5% agreement with the expectation.

Additionally, 52 upper respiratory samples were tested for both qPCR and digital PCR in conjunction using the same purified RNA sample. 11 samples were determined to be positive by qPCR, while 16 samples were determined to be positive by dPCR. The results also agreed with what was observed in scientific evidence and the analytical data generated by Gnomegen. As shown in table 2, there were 4 samples of patients with SARS-CoV-2, which went undetected by qPCR, but were right at the limit of detection for the Gnomegen RT-Digital PCR Detection kit.

Table 2: Comparator Data

Sample Type	Patient Status	Determined by qPCR kit	Determined by Gnomegen RT-Digital PCR Detection kit
Upper Respiratory Specimen	Negative	41*	37
	Positive	11	15*

Similarly, 14 lower respiratory samples were tested in both qPCR and digital PCR. Of the samples tested, 13 of them were in the presumptive grey area for qPCR. For dPCR, 2 out of 13 were determined to be positive. The one positive was still determined positive using dPCR.

Collectively using the data gathered, we believe that digital PCR assays are more sensitive than qPCR without observing significant issues in false positives. This observation is exemplified when comparing results generated from qPCR and dPCR using the CDC qPCR assays at the LoD of digital PCR,

the positive rate was 95% for dPCR and 62.5% for qPCR. Comparing qPCR to dPCR there is an increase of over 30% in sensitivity.

Note: All analytical data are done in comparison to CDC qPCR assays. All non-contrived clinical data were done in comparison to NMPA approved COVID-19 data used on the currently on the market in China.

REFERENCES:

^{1} Rabin, R.C., They were infected with Coronavirus. They Never Showed Signs. New York Times. 6, March 2020. <https://www.nytimes.com/2020/02/26/health/coronavirus-asymptomatic.html>

^{2} Wharton, J. Man Dies of coronavirus five days after being given the all-clear. Metro News. 6, March 2020. <https://metro.co.uk/2020/03/06/man-dies-coronavirus-five-days-given-clear-12359243/>