

## LETTER TO THE EDITOR

# RT-PCR Test Based on Different Gene Target for COVID-19 Diagnosis: a Reappraisal for low Viral Quantity Situation

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Molecular diagnosis is required for confirmation for COVID-19 [1]. A standard RT-PCR test is widely used at present. There are many molecular diagnostic assays available based on different target genes of the virus, including E, N, ORF1a/ab, RdRp, and S genes. The different molecular diagnostic assays have different cutoff values and different diagnostic properties. How the difference can affect the diagnosis is an interesting question. An important consideration is the false negative, which might result in under diagnosis of an infected case, and there is a chance of further disease spreading. According to a period evaluation study on 41 available assays in our setting [2], assays with problems of false positive and false negative results are equal to 5% and 27%, respectively. Hence, the problem of false negative RT-PCR test for COVID-19 should be highlighted. The special focus should be given to the situation that there is a low viral quantity. Here, the authors reappraise the cycle threshold in diagnosis of sample with low viral quantity less than 500 copies/mL. Results from the reappraisal are presented in Table 1.

Based on the reappraisal, observed cycle threshold level for assays with different target genes are different. Assay with ORF1a/ab target has the best sensitivity for detection of low viral quantity followed by an assay with RdRp target. Regarding assay specific cutoff cycle threshold level, if an assay, regardless of target gene, with a good threshold limit is selected, it can still permit an accurate diagnosis. On the other hand, if an assay

**Table 1. Observed cycle threshold in diagnosis of sample with low viral quantity from reappraisal on different RT-PCR tests.**

Gene targets	Observed cycle threshold level (range)	Assay specific cutoff cycle threshold level (range)
E	32.9 - 37.4	35 - 45
N	17.4 - 38.8	35 - 41
ORF1a/ab	23.6 - 40.0	37 - 41
RdRp	32.7 - 39.6	35 - 45
S genes	33.6 - 36.6	36 - 37

Numbers of available assays with E, N, ORF1a/ ab, RdRp, and S gene targets for evaluation by triplicate analysis are equal to 13, 22, 19, 13, and 4, respectively.

with a good threshold limit is selected, the chance of false negative will be highest for the assay with RdRp, followed by N, ORF1a/ab, E, and S gene target, orderly.

**Declaration of Interest:**

None.

**References:**

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