Mutation at the Cdc Sars-Cov-2 Diagnose Primer-Probe Assay Binding Sites

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ABSTRACT

The present outbreak of SARS-CoV-2 infection has become a pandemic according the World Health Organization. However, the current therapeutic strategies to deal with the infection are only supportive. And the effective vaccine against SARS-CoV-2 is still under development. So the prevention aimed at reducing transmission in the community is our top-priority. The precise diagnosis to discriminate the person who carries the SARS-CoV-2 virus is the key to control the spread of the infections.

INTRODUCTION

The current diagnosis method promoted by the Centers for Disease Control and Prevention (CDC) based on the PCR primer-probe binding to the reference genome of the SARS-CoV-2 virus[1]. There are three primer-probe sets (N1, N2, N3) designed to bind to the genomic region coding for protein nucleocapsid. However, a general characteristic of RNA viruses is the high rate of genetic mutation, which leads to new viral strains and hinders the annealing between primer and template if the mutation happened at the binding sites. Therefore, it is important to check whether there is new SARS-CoV-2 mutation at the primer-probe binding sites [1,2].

By align the 890 complete genomes released from the Global Initiative on Sharing All Influenza Data (GISAID) database till 20/03/2020, I found 8 out of 9 primer-probe binding sites contain single nucleotide mutation. Only the binding site of the forward primer of N2 has not found any mutation. The mutations at the N1 and N2 primer-probe binding sites are found in low frequency, found only once for each mutation. However, the mutations at the N3 primer-probe binding sites are found in high frequency. The N3 forward and reverse primers, in particular, are found in about 5% and 0.6% of sequenced genomic samples. A cumulative of virus samples with these two mutations is founded during the period pandemic development (Figure 1) [3-5].

Figure 1: A) The accumulation of the mutated genomic samples at the N3 forward primer binding site (genome position: 28739) over the time period from 01/01/2020 to 20/03/2020. B) The accumulation of the mutated genomic samples at the N3 reverse primer binding site (genome position: 28739) over the time period from 01/01/2020 to 20/03/2020.

In addition, the mutation frequency at the N3 forward primer binding site (genome position 28688) is over 10% for the sample from Australia, New Zealand, Spain, and Kuwait, while the majority of the mutation at the N3 reverse primer are found within samples from Netherlands (Figure 2). So it is worth to note that the above country should use alternative primer-probe sets to replace N3 primer-probe set.

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Received: April 02, 2020; Accepted: August 24, 2021; Published: September 02, 2021

Citation: Yan Yan et al. (2021) Mutation at the Cdc Sars-Cov-2 Diagnose Primer-Probe Assay Binding Sites. J Infect Dis Diagn. 6:15

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Figure 2: C) The mutation frequency at genome position 28688 for the samples from different countries. D) The mutation frequency at genome position 28739 for the samples from different countries.

REFERENCES


