Review 2: "A rigorous framework for detecting SARS-CoV-2 spike protein mutational ensemble from genomic and structural features "

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RR:C19 Evidence Scale rating by reviewer:

- **Strong.** The main study claims are very well-justified by the data and analytic methods used. There is little room for doubt that the study produced has very similar results and conclusions as compared with the hypothetical ideal study. The study's main claims should be considered conclusive and actionable without reservation.

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Review:

The manuscript entitled "A rigorous framework for detecting SARS-CoV-2 spike protein mutational ensemble from genomic and structural features" has been reviewed. In this manuscript, the authors have tracked 3,11,795 genome sequences of the spike protein, which includes 2,584 protein mutations. Also, the authors have detected three novel mutational clusters, namely A222, N439, and S477. Additionally, by examining structural features from 44 known cryo-EM structures, the authors observed that the virus is deploying many mutations within these clusters on structurally heterogeneous regions. The manuscript has been written in a clear, precise, and easy to understand way. A lot of effort was put into this study. As far as I can determine, the work is solid and strong. Therefore, I think that the manuscript meets the general criteria for publication.