Effect of oropharyngeal swab quality on the success of NGS library preparation

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SARS-CoV-2 genomes rapidly change due to mutations, therefore, the fast evolution of this virus has been observed worldwide. As is known, the majority of the identified mutations do not influence a significant effect on the spread. While some mutations or combinations can provide the virus advantages because some strains spread quickly around local populations. We had tested 477 oropharyngeal swabs from patients from different regions of Russia with diagnosis COVID-19 to perform a whole-genome sequencing to detect new variants of SARS-CoV-2. We were faced with the problem of low-quality samples. It had led to the deterioration of amplification. It's a significant moment to perform screening tests.
Methods: Previously designed primers panel [1] was used for SARS-CoV-2 whole genome amplification. The PCR products ranging from 1757 to 2054 bp were mixed, purified. Libraries were constructed using Nextera XT DNA Library Preparation Kit (Illumina, FC-131-1096). Sequencing was performed as described in [1]. The consensus sequence was submitted to GISAID database (hCoV-19/Russia/CRIE).
Results: We completely amplified and whole-genome sequenced the 173 of SARS-CoV-2 genomes from 477 samples. The success of genome fragments amplification varied from 76% to zero when we used samples obtained from different sources (from different clinics). We suppose that this dramatic difference could be explained by the composition of transportation buffers that are purchased by different clinics for routine diagnostics of COVID-19 by RT-PCR methods. Our results demonstrated that the success of amplification does not depend on the storage and transportation time.
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References:
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