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DNA MACHINE FOR SARS-COV-2 DETECTION IN THE CLINICAL SAMPLES

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Despite the high sensitivity of the DNAzymes among other diagnostic constructs, it's still not satisfying for the viral diagnosis due to the insufficient LOD. In this study, we discuss a developed DNAzyme-based machine with LOD 0.1 pM. In a trial to enable competition with the RT-qPCR.

Introduction Viruses that contain ribonucleic acid are highly mutable. Therefore, it sometimes leads to fiercer symptoms. The early detection of viruses is the key to a fast and safe recovery and a way for epidemiological management. However, it is a challenge because of the cost and complexity of the detection process. These days, the quantitative PCR (qPCR) is dominating the majority of the analysis methods. This technique is well-known but still requires sophisticated equipment and reagents. Its throughput is limited by the number of wells in the device.

Main Part. The new generation of the DNAzymes (DNA Machines) is the light of hope that can make diagnostics of viral infections to be much simpler and lower in the cost. DNAzymes are showing promising development and success in the last few years starting from a developed probe to the binary DNAzyme (10-23). The DNAzyme (10-23) is a DNA oligo nucleotide that has a catalytic activity, for specific sequence due to its catalytic core that can be formed after binding to that sequence. Binary DNAzyme probe consists of two separated strands that attach to the analyte (the RNA sequence) to form the catalytic core followed by binding and cleavage fluorogenic reporter substrate (F-sub). The advantage of the binary DNAzyme that it can form the catalytic core not only in presence of the F-sub, but also it needs an input to be activated. This can help us to detect the viral RNA, and it has also a unique advantage that it amplifies the signal: one molecule of RNA analyte will allow one copy of the DNAzyme to cleave multiple F-sub molecules. Moreover, binary DNAzyme probe demonstrates improved selectivity of nucleic acid recognition in comparison with conventional hybridization probes. However, the binary DNA zyme (10-23) still has limitations with the sensitivity over the long analytes that form more stable secondary structures. We aimed to transform binary DNAzyme probe into a DNA machine by adding two additional binding arms, three of which are linked to a common double stranded DNA platform. The machine is able to unwind the secondary RNA structures by total of 4 binding arms. Sensitivity was successfully enhanced, and the Developed DNA-Machines showed high activity and sensitivity with limit of detection below 0.1 pM, which is 200 times lower than that of BiDz. This made it easier to try the viral detection directly in the clincal samples with no amplification step.

Applications.

The obtained results showed that we can detect the viral RNA in the clinical samples without any amplification step. This finding opens the door for the future substitution of the q-PCR and promises to turn the diagnostic process to be easier and cheaper.