УДК 577.29

MULTICORE DNA MACHINE BASED ON BINARY DNAZYME FOR THE AMPLIFICATION-FREE DETECTION OF SARS-COV-2

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Introduction. According to RBC, since the beginning of the coronavirus pandemic, more than 21 million cases of the disease have been registered in Russia [1]. The world market of laboratory diagnostics was not ready for the operational preparation and processing of a large flow of analyses, and therefore the cost of services and the waiting time for results increased. The search for new ways of coronavirus infection diagnostics will reduce the cost of the necessary analysis, as well as speed up and simplify the patient's entire path during treatment.

The main part. Binary deoxyribozymes, or DNAzymes, are a combination of synthetic single-stranded DNA oligonucleotides. These constructions can be considered as a promising method of diagnosing various diseases due to their high sensitivity, biocompatibility, stability, low cost of synthesis and ease of structure prediction [2]. The aim of this work is to develop an alternative method for the diagnosis of SARS-CoV-2 in patients using a deoxyribosime-based DNA machine without viral RNA amplification.

To achieve this goal, we have formulated the following tasks: 1) To develop an optimized design of the DNA machine capable of increasing the sensitivity of the analysis; 2) To determine the LOD of the DNA machine at three time points and prove the effectiveness of the design in comparison with the previous design of the DNA machine; 3) To check the LOD of each core separately. The following materials and methods were used in the work: a fluorescent approach, a fluorescent substrate (f-sub) at a concentration of 200 nM, DNA oligonucleotides, a reaction buffer with a concentration of Mg2+ 200 mM, gel electrophoresis in 2% agarose. The efficiency of the design was compared with the previously developed DNA machine with a single catalytic center [3].

The paper proposed a DNA machine design that includes four binary deoxyribozymes. Each DNAzyme contains analyte binding regions and sites of attachment to the f-sub. The fluorescent substrate contains a quencher (BHQ), which blocks the fluorescence from the fluorophore. In the presence of viral DNA in the test sample, four catalytic cores are formed in the DNA machine structure, respectively, which initiate f-sub cleavage and, accordingly, the BHQ is not able to block the fluorescence, which further allows detecting the signal using a spectrophotometer. Thus, the DNA machine is able to multiply the cleavage of signaling substrates after the detection of a single virus molecule.

Conclusion. During testing of the proposed design, concentrations of oligonucleotides included in the DNA of the machine were selected with minimal effect on the background fluorescence noise of the samples. The machine has been successfully assembled and tested on the synthetic analyte (SARS-CoV-2 DNA) in order to calculate the LOD (obtained LOD is 4-5 times less than the LOD of the previous design [3]); it was proved that the machine is capable to detect virus in a less time – 30 min. Also the LOD of each core was calculated: the results of different sensitivity of different cores make us to conclude about sequence dependence of the cores catalytic activity. Thus, in the course of the work carried out, the effectiveness of the DNA machine design under consideration was proved, and reducing the time to obtain the result opens up the prospect of using this design at home by the patient himself.

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