Draft of the Targeted Funding Program Project"Development of New Diagnostic Test Systems for Especially Dangerous Viral Infections"IRN: BR24992948

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Annotation

- Following the COVID-19 pandemic, global challenges related to the rapid diagnosis and treatment of emerging infectious diseases, as well as the development of measures to prevent their spread, became evident. Due to the broad range of genetic modifications, high variability, and the increasing logistics of international trade, viral infections pose a significant threat to Kazakhstan.
- This project aims to develop two diagnostic test systems. The first is a multiplex RT-PCR test for the laboratory diagnosis of SARS-CoV-2 and influenza A virus. The second is an ELISA-based test system for the detection of antibodies against the SARS-CoV-2 pathogen.
- Implementation of the developed diagnostic tools will include laboratory testing, registration, and patenting. Research results will be published in scientific journals. The diagnostic tools created will provide a platform for the development of domestic test systems for other infectious diseases.

Goal

- The goal of this study is to develop diagnostic test systems for the differential detection of influenza and coronavirus infection using multiplex real-time RT-PCR, and to assess the population's immune status by detecting antibodies to SARS-CoV-2 through ELISA.

План публикаций работ

- In 2025, one article is planned for publication in a journal recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education (KOKSNVO), and two patent applications will be submitted to the National Institute of Intellectual Property.
- In 2026, one article is planned for publication in a peer-reviewed scientific journal.

Tasks

Development and testing of a real-time RT-PCR-based diagnostic system for the detection of influenza and SARS-CoV-2 RNA:

- Design of primers and probes;
- Development of positive and negative control samples;
- Optimization of reagent mixture composition and thermal cycling conditions;
- Determination of the specificity and sensitivity of the test system;
- Laboratory testing and validation of the diagnostic system;
- Commissioned testing of the test system;
- Development of regulatory documentation (NTD) for the test system;
- Implementation of diagnostic tools for especially dangerous viral infections by obtaining registration certificates;
- Registration of the test system for the detection of influenza and coronavirus infection.

Development and testing of a diagnostic tool for the detection of antibodies against the SARS-CoV-2 pathogen via ELISA:

- Production of recombinant antigens and specific sera, and optimization of assay conditions;
- Determination of the specificity and sensitivity of the diagnostic test system for antibody detection;
- Laboratory testing of the ELISA test system for antibody detection;
- Determination of the clinical specificity and sensitivity of the test system;
- Commissioned testing of the ELISA test system;
- Development of regulatory documentation for the ELISA test system;
- Registration of the antibody detection test system.

Methods

- Multiple sequence alignment of nucleotide sequences;
- Design and specificity analysis of oligonucleotide primers and probes;
- Viral RNA extraction;
- Construction of recombinant plasmid DNA;
- RT-PCR and real-time RT-PCR setup;
- Optimization of RT-PCR analysis and evaluation of test system specificity and sensitivity;
- Construction of expression plasmids for recombinant proteins;
- Expression of recombinant proteins;
- SDS-PAGE electrophoresis and Western blotting;
- Production of specific hyperimmune sera.

Results and discussion

Current results of the research include:

- Based on alignment and analysis of the nucleotide sequences of influenza A and SARS-CoV-2 genomes, conserved and polymorphic regions were identified and used to design species-specific primers and probes for real-time RT-PCR.
- Specific primers and probes were selected and synthesized for the diagnosis of influenza A virus and SARS-CoV-2 by real-time RT-PCR.
- The quantitative composition of reagents, oligonucleotide probes, and primers in the reaction mixture was optimized, and the optimal temperature profile for PCR amplification was selected using gradient RT-PCR.
- Positive controls for influenza A and SARS-CoV-2 viruses were obtained.
- A recombinant plasmid was constructed for the expression of a chimeric polyepitope protein.
- Immunospecific components were produced: recombinant protein and hyperimmune sera for developing a test system for detecting antibodies to SARS-CoV-2 in blood serum.
- Optimal ELISA conditions were determined.

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