ANALYSIS OF THE VARIABILITY OF GENES FOR HEMAGGLUTININ, NEUROMINIDASE AND NUCLEOPROTEIN OF AVIAN INFLUENZA STRAINS *h1n1* AND *h7n9*. IDENTIFICATION METHOD PCR COMBINED WITH RFLP

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The active evolutionary process in some groups of infectious agents, combined with the activation of migration flows in human populations, creates the prerequisites for the emergence and global spread of infectious diseases. At present, the spread of epizootics of highly pathogenic avian influenza in the world is of considerable concern. Multiple outbreaks caused by the *H5, H7,* and *H9* virus subtypes in wild birds have given rise to observations of avian influenza viruses in various regions around the world.

The aim of the research was to create a system for epidemiological monitoring of the circulation of a highly pathogenic avian influenza virus of the "wild" strain *h7n9* and *h1n1* based on the test systems developed by us for molecular diagnostics and genotyping.

Based on the analysis of hemagglutinin, neuraminidase, and nucleoprotein gene polymorphisms, variable pairs of oligonucleotides specific for the *H1N1* and *H7N9* viral subtypes were obtained. More than 8,000 influenza A, *NA*, and *NP* gene sequences of the *H1N1* and *H7N9* subtypes, allotted by 2017, were used for primer selection. The sequences were analyzed using Alignment Service and Lasergene *(version 6.0)*. The homology level of the selected primers is not less than 95%. The method involves conducting a polymerase chain reaction with RNA virus combined with the amplification reaction for three genes, analysis of the reaction mixture by agarose gel electrophoresis and detection of RNA strains of influenza A viruses by analysis. The first is the PCR with primers specific to the site of the influenza A hemagglutinin gene. The second reaction is RFLP with endonucleases specific for the hemagglutinin gene, neuraminidase and nucleoprotein regions. Identification of the strain *H1N1*, the samples of which form in RFLP analysis using good restriction enzymes to the gene *NP* unique products sizes 49-50, 348-350, 592-599 BP, others – fragment amplification sizes 21, 39, 201-203, 471-480 BP, identical to the products of RFLP analysis using good restriction enzyme strain *H7N9*. The *in silico* analysis of the *HA, NA* and *NP* gene amplicons allowed us to obtain theoretical PCR-RFLP electrophoregrams of the analysis, to calculate the reaction conditions, to determine restriction sites in matched restriction enzymes.

The developed method of express identification based on PCR combined with RFLP analysis makes it possible to significantly simplify the identification method due to the specific amplification of the RNA region with a polymorphic restriction site. The state testing of this locus is possible by preliminary PCR and restriction of the amplified fragment. It was established that the PCR-RFLP express diagnostic method was capable of detecting the RNA of influenza A virus of highly pathogenic *H1N1* and *H7N9* strains with high sensitivity indicators (100% sensitivity).