SELECTION OF PRIMERS FOR THE SUBUNITS OF HEMAGGLUTININ, NEURAMINIDASE AND NUCLEOPROTEIN SUBTYPES OF H1N1 AND H7N9 OF INFLUENZA A

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Influenza A virus belongs to the family *Orthomyxoviridae*. These viruses infect many species of wild and domestic birds, mammals and humans. Currently, there is a prevalence of the highly pathogenic avian influenza virus H1N1 and H7N9. Influenza A virus genome contains eight negatively charged glycoproteins. Hemagglutinin and neuraminidase have a high level of genetic variation. The evolution of influenza A virus proceeds very quickly, therefore, the primary factor is the analysis of the polymorphism of the hemagglutinin genes of those subtypes, as well as the identification of antigenic structure. The aim of our work was to select *in silico* primers and optimize the conditions for PCR amplification of the HA, NA, and NP genes and their subunits for subsequent work on the creation of the PCR - RFLP method for rapid identification of subtypes H1N1 and H7N9 of type A avian influenza virus. The primers were constructed using the Lasergene bioinformation programs (version 6.0, https://www.dnastar.com/software/molecular-biology), BioEdit (version 7.00, https://bioedit.software.informer.com/7.0) and the NCBI scientific database (National Center for Biotechnological Information, USA). The design of the primers was preceded by a preliminary stage of studying the variability of the hemagglutinin, neuraminidase, and nucleoprotein genes of the studied strains of the influenza A virus. Given the large variability of the influenza A genome, it is necessary to use primers that identify the most conserved regions of the viral genome. After an *in silico* analysis of amplicons of the HA, NA, and NP genes, HA5 hemagglutinin primers – AGACCCAAGGTGAGAGGTCA and AGAAAACTGATTGCCCCCAGG were designed. Primers for HA10 were GCCGCAAATGCAGACACATT and GCTGCCGTCACACACCTCTATT. Primers specific for the NA1 gene region were CAGGAGCCCATATCGAACCC and CTTTGGGTCGCCCTCTGATT. For the NA8 gene: - TGCAGGGATAACTGGCATGG and GCTCCCGCTAGTCCAGATTG. The third primer pair was obtained for the NP5 gene: - CTGGTCAGCCTGATGAGACC and GGGTTCGTTGCCTTTTTCGTC. Primers showed complementary to at least 95% of the hemagglutinin, neuraminidase and nucleoprotein gene. The length of the PCR products was 958 bp for the HA5 gene (H1N1) and 966 bp for (H7N9). For HA10 (H1N1), 416 bp and for H7N9 411 bp were obtained. For NA1 (H1N1) and H7N9 strains were 845 bp and 848 bp, respectively. At least for NA8 (H1N1) and H7N9 were 450 bp and 447 bp, respectively.

The pairs of primers were designed for the influenza A genes HA, NA, and NP of the H1N1 and H7N9 subtypes, which are highly conserved and specific for its subtype and does not have homology with other subtypes of influenza A virus.