DEVELOPMENT OF A HIGHLY SENSITIVE 4-TH GENERATION ELISA TEST KIT FOR THE DETECTION OF HEPATITIS C VIRUS

Horlov A, Serdyuk V, Chumak E, Spivak N.
PJSC «SPC «Diaproph-Med»,
Research and Development Department
e-mail: an.gorlov@gmail.com

Hepatitis C virus (HCV) is the major causative agent of transfusion-associated hepatitis. The HCV genome is represented by a single-stranded, positive sense RNA of approximately 9600 nucleotides encoding for a polyprotein of 3011 aa, which is then processed into structural proteins – core (C), E1, E2 and non-structural proteins, including NS2, NS3, NS4a, NS4b, NS5a, and NS5b. Among the structural proteins, core antigen is highly conserved and induces a rapid Ab response and is one of the earliest markers of HCV infection.

The 1st generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Earlier generation tests, which included recombinant/synthetic antigens from the core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. The 3rd generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). The fourth-generation assays involve biotin-streptavidin amplification of the specific signal and provide the highest sensitivity. The main goal of this work was to establish highly immunoreactive peptide constructs, which could be used for development of the new high sensitivity 4th generation HCV ELISA test. To this aim we have designed 4 peptides based on the sequences of HCV core and NS4 antigens. Biotinylated peptides were evaluated by ELISA to detect anti-HCV antibodies. As a result, the new test, utilizing designed peptides, could detect more low-titer HCV-positive serum samples and achieve much higher sensitivity compared with the 3rd generation ELISA HCV tests.