## P1401

## **Poster Session V**

Molecular and non-molecular diagnostics of viruses

INFLUENCE OF HETEROGENEITY OF AMINO ACID SEQUENCE VARYING CONSIDERABLY ON IMMUNOREACTIVITY ANTIGEN REGION OF HCV NS5 PROTEIN FROM POSITION 2212 TO 2313

G. Bochkova<sup>1</sup>, T. Ulanova<sup>2</sup>, V. Puzyrev<sup>3</sup>, A. Burkov<sup>4</sup>

<sup>1</sup>HCV, RPC "Diagnostic Systems', Nizhniy Novgorod, Russia; <sup>2</sup>DSI S.R.L. Saronno, RPC "Diagnostic Systems", Nizhniy Novgorod, Russia; <sup>3</sup>Department for production of recombinant antigens and conjugates, RPC "Diagnostic Systems", Nizhniy Novgorod, Russia; <sup>4</sup>General Director, RPC "Diagnostic Systems", Nizhniy Novgorod, Russia

There are a very little data regarding the influence of genetic heterogeneity of NS5 protein on the efficiency of antibody detection.

**Aim:** The purpose of this study was evaluating diagnostic relevance of sequence heterogeneity of HCV NS5 proteins.

**Objectives and methods:** 9 different synthetic genes encoding epitopes of HCV NS5A proteins containing HCV regions at position 2212 -2313 aa of genotypes 1a, 2a, 2b, 3a, 3b, 4a, 5a, 6a were assembled by PCR from oligonucleotides and expressed as fusion proteins with Glutathione Stransferase in E. coli. Immunoreactivity of these artificial proteins was evaluated by testing well defined anti-HCV positive (n=80) sera samples with different genotypes obtained from patients infected with HCV from Russia and anti-HCV negative (n=82) samples in ELISA.

**Results:** All proteins have very different immunoreactivity. NS5-1 (1a) had the most level of immunoreactivity and detected anti-HCV in 60.0 % of positive samples. NS5-3 (2b) had the lowest immunoreactivity and detected anti-HCV in 2.5 % of samples. NS5-2 (2a) detected anti-HCV in 51% of samples, NS5-4 (3b) – in 40.6%, NS5-5 (2a) – in 45%, NS5-6 (4a) – in 42.5%, NS5-7 (6a) – in 6.25%, NS5-8 (3a) – in 18.75%, NS5-9 (5a) – in 43.75%. Complex of three proteins NS5-1 (1?), NS5-5 (2a), NS5-8 (3a) made possible to detect anti-HCV in 75 % of positive samples, complex of four proteins NS5-1 (1a), NS5-2 (2a), NS5-5 (2a) and NS5-8 (3a) – in 80%.

**Conclusion:** These data demonstrate that variation in the primary structure has a significant effect on the antigenic properties of the HCV NS5 protein and should be taken into a consideration when selecting diagnostic target for the development of highly specific and sensitive diagnostic assays for the detection of anti-HCV activity in sera specimens.