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P0789 Modification of a recombinant antigen results in improved diagnostic value for enzyme immunoassay detection of anti-hepatitis C antibodies

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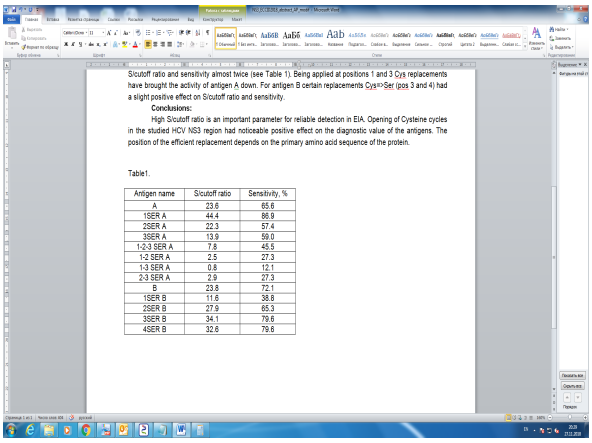
Background: Presence of hepatitis C virus (HCV) nonstructural protein 3 (NS3) in primary detection of anti-HCV IgG in enzyme linked immunoassay (ELISA) is an acting requirement of modern tests. A part of HCV NS3 antigen region 1357–1459 amino acids (aa) was shown to be immunodominant and conformational. Correct formation of S-S bonds is essential for high antigen sensitivity.

Evaluation of diagnostic relevance of modified artificial proteins originated from 1357–1459 aa of HCV NS3 antigen by enzyme immunoassay (EIA) for the detection of anti-NS3 IgG activity in serum specimens has been studied.

Materials/methods: Region coding 1357-1459 aa of HCV NS3 sequence was amplified from HCV positive serum and has been cloned as a GST-fusion protein. Two of the obtained recombinant antigens were modified by side-directed mutagenesis. In sequence A - three available Cysteine residues (Cys) have been replaces with Serine (Ser), in sequence B – four. 13 new constructs were expressed in *Escherichia coli* strain and purified by affinity chromatography. To evaluate influence of Cys-Ser replacement on the ability to detect anti-HCV antibodies the new antigens have been tested against well-known 61 anti-HCV positive and 27 anti-HCV negative serum specimens in a format of newly developed EIA.

Results: For antigen A opening of Cysteine cycle at position 1 had crucial effect and increased S/cutoff ratio and sensitivity almost twice (see Table 1). Being applied at positions 1 and 3 Cys replacements have brought the activity of antigen A down. For antigen B certain replacements Cys=>Ser (pos 3 and 4) had a slight positive effect on S/cutoff ratio and sensitivity.

Conclusions: High S/cutoff ratio is an important parameter for reliable EIA-detection. Opening of Cysteine cycles in the studied HCV NS3 region had noticeable positive effect on the diagnostic value of the antigens. The position of the efficient replacement depends on the primary amino acid sequence of the protein.


S/cutoff ratio and sensitivity almost twice (see Table 1). Being applied at positions 1 and 3 Cys replacements have brought the activity of antigen A down. For antigen B certain replacements Cys=>Ser (pos 3 and 4) had a slight positive effect on S/cutoff ratio and sensitivity.

Conclusions:

High S/cutoff ratio is an important parameter for reliable detection in EIA. Opening of Cysteine cycles in the studied HCV NS3 region had noticeable positive effect on the diagnostic value of the antigens. The position of the efficient replacement depends on the primary amino acid sequence of the protein.

Table1.

Antigen name	S/cutoff ratio	Sensitivity, %
A	23.6	65.6
1SER A	44.4	86.9
2SER A	22.3	57.4
3SER A	13.9	59.0
1-2-3 SER A	7.8	45.5
1-2 SER A	2.5	27.3
1-3 SER A	0.8	12.1
2-3 SER A	2.9	27.3
B	23.8	72.1
1SER B	11.6	38.8
2SER B	27.9	65.3
3SER B	34.1	79.6
4SER B	32.6	79.6