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 the 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 (see Picture 1). 13 new constructs were expressed in *Escherichia coli* strain and purified by affinity chromatography, the purity of the proteins was verified by SDS-PAGE. 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. ELISA format has included incubation with serum samples for 1h on shaker at 37C, divided from reaction with conjugate for 1h (mouse anti-Human IgG-HRP) by 3 washes with PBST; incubation with TMB took 20 min, the reaction was stopped by 0,2M H2SO4. Optical density was measured at wavelengths 450-620nm.

Picture 1

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). For antigen A replacement at position 1 had the best effect on the original sequence. Being applied at positions 1 and 3 in antigen A, Cys replacements have brought the activity of antigen A down.

For antigen B replacements Cys=>Ser at positions 3 and 4 had a positive effect on S/cutoff ratio and sensitivity. Mutations Cys=> Ser in positions 1 double decreased performance indexes.

Table 1

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

Conclusion

High S/cutoff ratio is an important parameter for reliable EIA-detection. Opening of Cysteine cycles in t he 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.