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(54) Titre : SEQUENCES D'UNE PROTEINE CAPSIDIQUE DU VHC
(54) Title: HCV CORE PROTEIN SEQUENCES

(57) Abrégé/Abstract:
The present invention describes peptides and recombinant proteins containing Hepatitis C virus core protein sequence in which one or more of the amino acids have been modified or deleted to remove the ability of these proteins to bind to specific anti-HCV murine monoclonal antibodies. The deletions and modifications are designed as to maintain the ability of this protein to be used in immunoassays used for the detection of anti-HCV antibodies in individuals infected with HCV.

Abstract

The present invention describes peptides and recombinant proteins containing Hepatitis C virus core protein sequence in which one or more of the amino acids have been modified or deleted to remove the ability of these proteins to bind to specific anti-HCV murine monoclonal antibodies. The deletions and modifications are designed as to maintain the ability of this protein to be used in immunoassays used for the detection of anti-HCV antibodies in individuals infected with HCV.

HCV Core Protein Sequences

Background of the Invention

An estimated 170 million people worldwide have been infected by hepatitis C virus (HCV). In the next few years, the number of U.S. deaths for HCV-caused liver disease and cancer may overtake deaths caused by Acquired Immune Deficiency Syndrome (AIDS).

The transmission of HCV seems to require blood-to-blood contact. Carrying a single strand of ribonucleic acid (RNA), HCV contains just one gene, coding for a polyprotein that is subsequently cleaved into at least 10 functional proteins. Clearly, the ability to test the blood supply for HCV is of great importance. A sensitive assay that can detect infection at an early stage would be helpful.

HCV detection assays typically detect antibodies against HCV virus. These antibodies are detected in immunoassays using recombinant proteins and peptides containing HCV protein sequences. Most commercial anti-HCV assays use proteins from the following regions, core protein, NS3,NS4,and NS5 protein sequences.

Anti-HCV core antibodies are one of the most prevalent anti-HCV antibodies detected in chronic HCV

infected individuals. HCV core protein contains multiple epitopes. Using synthetic peptides in the HCV core region, it has been shown that most of these epitopes are in the amino terminal end of this protein.

For example, using overlapping peptides, each approximately 15 amino acids in length, an ELISA was developed to screen for anti-HCV antibodies in chronic HCV infected individuals. Table 1 below shows the peptide sequences. As shown in Table 2 below, HCV infected individuals have antibodies to two or more of these core peptides. Thus, it has been shown that the complete core peptide is not required to detect anti-core antibodies.

Table 1

Peptide ID #	Amino Acid Sequence	HCV Polyprotein AA Location
0	MSTNPKPQKKNKRNT	1-15 SEQ ID NO.:1
1	KNKRNTNRRPQDVKF	10-24 SEQ ID NO.:2
2	QDVKFPGGGQIVGGV	20-34 SEQ ID NO.:3
3	QIVGGVYLLPRRGPR	29-43 SEQ ID NO.:4
4	RRGPRLGVRATRKTS	39-53 SEQ ID NO.:5
5	ATRKTSERSQPRGRR	48-62 SEQ ID NO.:6
6	PRGRRQPIPKARRPE	58-72 SEQ ID NO.:7
7	KARRPEGRTWAQPGY	67-81 SEQ ID NO.:8
8	AQPGYPWPLYGNEGC	77-91 SEQ ID NO.:9
9	YGNEGCGWAGWLLSP	86-100 SEQ ID NO.:10
10	WLLSPRGSRPSWGPT	96-110 SEQ ID NO.:11
11	SWGPTDPRRRSRLNG	106-120 SEQ ID NO.:12
12	SRLNGKVIDTLTCGF	116-130 SEQ ID NO.:13
13	LTCGFADLMGYIPLV	126-140 SEQ ID NO.:14
14	YIPLVGAPLGGAARA	136-150 SEQ ID NO.:15
15	GAARALAHGVRVLED	146-160 SEQ ID NO.:16
16	RVLEDGVNYATGNLP	156-170 SEQ ID NO.:17
17	TGNLPGCSFSIFLLA	166-180 SEQ ID NO.:18

Table 2

Peptide	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Neg	0.044	0.004	0.021	0.075	0.059	0.069	0.076	0.103	0.020	0.034	0.002	0.088	0.051	0.028	0.023	0.034	0.041	0.042
Neg	0.045	0.065	0.017	0.081	0.051	0.081	0.103	0.111	0.033	0.035	0.002	0.085	0.038	0.030	0.023	0.030	0.038	0.052
Neg	0.050	0.003	0.014	0.075	0.087	0.079	0.094	0.102	0.025	0.034	0.002	0.071	0.038	0.033	0.021	0.031	0.039	0.047
Neg	0.014	0.043	0.134	0.023	0.034	0.039	0.034	0.039	0.012	0.018	-0.001	0.025	0.009	0.009	0.008	0.013	0.010	0.020
Neg	0.015	0.039	0.119	0.021	0.033	0.030	0.030	0.034	0.013	0.019	0.000	0.026	0.011	0.010	0.005	0.012	0.010	0.016
Neg	0.013	0.036	0.126	0.027	0.053	0.032	0.037	0.042	0.013	0.016	0.001	0.037	0.013	0.009	0.009	0.012	0.009	0.017
Neg	0.016	0.004	0.104	0.020	0.039	0.034	0.003	0.041	0.019	0.019	0.000	0.041	0.011	0.017	0.006	0.010	0.008	0.021
9	0.256	2.500	2.500	0.425	1.443	0.106	0.218	0.248	0.062	0.015	0.025	0.049	0.030	0.013	0.012	0.021	0.012	0.028
11	0.413	2.500	2.500	0.917	0.229	0.647	0.114	0.262	0.166	0.159	0.002	0.055	0.022	0.027	0.021	0.042	0.247	0.088
15	0.044	0.661	2.500	0.067	0.577	0.057	0.110	0.072	0.061	0.032	-0.003	0.045	0.018	0.031	0.020	0.039	0.035	0.080
18	1.643	0.887	2.500	0.641	0.100	0.052	0.032	0.576	0.037	0.022	-0.001	0.040	0.006	0.010	0.009	0.025	0.036	0.039
20	0.021	0.111	2.500	2.500	0.482	0.032	0.029	0.045	0.028	0.015	0.011	0.035	0.009	0.014	0.009	0.017	0.019	0.059
21	2.500	2.500	2.500	2.262	2.500	0.516	2.259	0.080	0.378	0.018	0.022	0.064	0.013	0.024	0.017	0.024	0.135	0.035
27	0.032	2.500	2.500	0.089	2.500	1.652	1.349	0.043	0.039	0.034	0.033	0.026	0.012	0.018	0.013	0.028	0.287	0.008
30	2.500	2.500	2.500	2.500	0.183	0.044	0.075	0.993	0.078	0.035	0.028	0.190	0.023	0.025	0.022	0.028	0.042	0.043
35	0.022	0.278	0.912	0.031	0.036	0.116	0.079	0.049	0.062	0.010	0.005	0.026	0.005	0.006	0.009	0.020	0.007	0.020
36	0.059	0.129	2.500	0.090	0.131	0.060	0.093	0.148	0.090	0.037	0.003	0.050	0.018	0.060	0.077	0.062	0.129	0.079
37	2.500	2.500	2.500	0.219	0.130	0.090	0.169	0.223	0.147	0.040	0.018	0.065	0.016	0.051	0.051	0.025	0.080	0.110
39	0.165	2.500	2.500	2.500	0.124	1.217	2.351	2.500	0.397	0.056	0.015	1.319	0.017	0.050	0.056	0.041	0.200	0.190
40	0.058	2.500	2.500	2.500	0.098	0.066	0.060	0.755	0.093	0.024	0.000	0.059	0.013	0.028	0.017	0.032	0.068	0.101
42	0.022	0.043	2.500	0.031	0.023	0.150	0.018	0.036	0.023	0.011	-0.001	0.032	0.005	0.014	0.006	0.010	0.012	0.031
43	0.158	0.612	0.368	0.033	0.065	0.103	0.069	0.075	0.053	0.051	0.001	0.055	0.025	0.022	0.015	0.023	0.029	0.035
44	0.042	2.240	2.500	0.511	0.070	0.060	0.086	0.301	0.108	0.066	-0.002	0.143	0.028	0.041	0.028	0.047	0.061	0.076

CA 02408174 2002-11-06

Peptide	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
45	1.002	2.500	2.500	0.174	0.713	0.055	0.129	1.722	1.896	0.029	-0.003	0.069	0.038	0.038	0.055	0.022	0.136	0.039
46	0.016	0.031	2.500	0.022	0.021	0.021	0.038	0.035	0.031	0.027	-0.002	0.027	0.016	0.015	0.013	0.026	0.019	0.037
47	2.484	2.500	2.500	0.449	0.750	0.593	0.102	2.006	0.331	0.017	-0.004	0.034	0.012	0.014	0.007	0.026	0.014	0.032
48	0.427	0.201	2.500	0.364	0.356	0.038	0.049	0.070	0.027	0.029	-0.001	0.040	0.018	0.011	0.013	0.025	0.014	0.095
49	0.019	0.483	2.500	0.044	0.045	0.047	0.050	0.246	0.039	0.025	-0.005	0.033	0.019	0.014	0.013	0.030	0.016	0.085
50	0.668	2.500	2.500	0.568	1.233	0.277	0.154	2.500	0.882	0.024	-0.001	0.035	0.014	0.012	0.007	0.017	0.044	0.055
52	2.500	2.500	2.500	0.948	1.116	1.619	0.026	0.267	0.112	0.019	0.026	0.042	0.012	0.007	0.009	0.019	0.008	0.044
53	0.823	2.500	2.500	0.428	2.500	0.780	0.086	2.500	1.566	0.025	0.001	0.050	0.014	0.020	0.011	0.022	0.019	0.028
54	1.577	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	0.020	0.032	0.053	0.021	0.012	0.012	0.020	0.017	0.038
55	0.017	0.083	2.500	0.047	0.018	0.023	0.071	0.042	0.019	0.017	-0.002	0.026	0.015	0.015	0.012	0.026	0.014	0.030
58	2.500	2.500	2.500	2.283	2.500	2.500	2.500	2.500	0.810	0.037	0.073	0.067	0.018	0.019	0.021	0.025	0.159	0.059
59	0.015	1.805	2.500	0.023	2.500	0.019	0.180	0.038	0.070	0.039	0.027	0.017	0.012	0.015	0.018	0.015	0.023	0.049
60	0.053	2.500	2.500	0.255	2.500	0.102	1.266	0.666	2.500	0.034	-0.003	0.153	0.026	0.019	0.022	0.039	0.097	0.063
62	0.018	0.053	2.500	1.178	0.025	0.031	0.041	0.060	0.028	0.017	-0.002	0.039	0.013	0.012	0.010	0.017	0.010	0.048
63	0.009	0.016	0.027	0.012	0.017	0.013	1.225	0.028	0.012	0.017	-0.004	0.026	0.007	0.007	0.006	0.010	0.057	0.020
65	1.440	0.041	0.026	0.040	0.032	0.056	0.052	0.062	0.020	0.040	0.023	0.051	0.018	0.023	0.023	0.019	0.020	0.035
66	2.060	2.500	2.500	1.030	2.500	0.508	0.144	2.500	2.500	0.013	0.009	0.060	0.007	0.009	0.009	0.014	0.025	0.018
67	2.500	2.500	2.500	2.500	2.500	1.338	0.406	2.500	0.135	0.031	0.003	0.091	0.021	0.027	0.028	0.032	0.042	0.077
68	2.500	2.500	2.500	1.105	0.415	0.358	1.025	2.500	0.263	0.028	0.006	0.039	0.023	0.025	0.022	0.024	0.034	0.037
70	0.745	2.500	2.500	0.342	2.500	0.200	0.049	0.075	1.730	0.030	0.009	0.119	0.015	0.025	0.022	0.027	0.056	0.050
73	0.031	2.392	2.500	0.049	0.629	0.935	0.596	1.119	0.081	0.040	0.009	0.052	0.028	0.036	0.031	0.038	0.043	0.059
75	0.037	2.500	2.500	1.435	1.342	0.107	0.287	0.094	0.080	0.049	0.012	0.031	0.013	0.030	0.017	0.021	0.058	0.050
78	2.500	2.500	2.500	0.269	2.500	0.298	0.051	2.500	0.040	0.057	0.006	0.084	0.013	0.022	0.014	0.021	0.022	0.030
80	0.280	2.500	2.500	0.674	0.318	0.972	0.048	2.118	0.040	0.021	0.022	0.024	0.015	0.010	0.008	0.014	0.066	0.031
84	0.012	0.075	2.500	0.025	0.190	0.145	0.106	0.099	0.019	0.028	0.050	0.018	0.009	0.010	0.009	0.013	0.009	0.026
90	0.019	0.306	2.500	0.039	2.500	0.689	0.033	0.301	0.195	0.021	0.005	0.033	0.011	0.017	0.010	0.013	0.014	0.018

A recent report indicates that the HCV core protein can be detected in HCV infected individuals before the appearance of anti-HCV antibodies. (S. Lee et al., Vox Sanguinis, 2001; 80: 19-23). Therefore, we suggest that a more efficient way of early detection of HCV infection would be a combination assay able to detect HCV core protein and anti-HCV antibodies including anti-core antibodies.

Summary of the Invention

The present invention describes peptides and recombinant proteins containing Hepatitis C virus core protein sequence in which one or more of the amino acids have been modified or deleted to remove the ability of these proteins to bind to specific anti-HCV murine monoclonal antibodies. These modifications were made in the underlined regions shown in Table 3 below. The deletions and modifications are designed as to maintain the ability of this protein to be used in immunoassays used for the detection of anti-HCV antibodies in individuals infected with HCV. These

modified core proteins can be used in a combination assay for the simultaneous detection of HCV core protein and anti-HCV antibodies. The combination assay will be able to detect HCV earlier than the currently used antibody assays.

In one embodiment there is provided a combination for the simultaneous detection of hepatitis C virus (HCV) antibody and HCV antigen comprising:

A) a solid phase coated with

1) an HCV core peptide, comprising the amino acid sequence of SEQ ID NO:19, wherein at least two of the following amino acid segments have one or more amino acids deleted,

i) MSTNPKPQRK (residues 1-10);

ii) IVGGVYLL (residues 30-37);

iii) RLGVRATR (residues 43-50); and

iv) AQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTD PRRRSRNLGKVIDTLTCGF (residues 77-130);

2) an HCV non-core protein, and

3) at least one anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind with the HCV core peptide described in A) 1); and

B) a labeled anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind to the HCV core peptide described in A) 1) and does not bind to the same epitope as the anti-HCV core monoclonal antibody described in A) 3).

In another embodiment, there is provided an isolated HCV core peptide comprising the amino acid

sequence of SEQ ID NO.: 19, wherein at least two of the following segments of amino acids have one or more amino acids deleted,

i) MSTNPKPQRK (residues 1-10);

ii) IVGGVYLL (residues 30-37);

iii) RLGVRATR (residues 43-50); and

v) AQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLGKV IDTLTCGF (residues 77-130),

and wherein amino acids 11-29, 38-42, and 51-76 are not deleted or altered.

In another embodiment there is provided a combination for the simultaneous detection of hepatitis C virus (HCV) antibody and HCV antigen comprising:

A) a solid phase coated with

1) an HCV core peptide, comprising the amino acid sequence of SEQ ID NO:19, wherein amino acids 1-8 MSTNPKPQ of SEQ ID NO:19 have been deleted and amino acids 31-33 VGG of SEQ ID NO:19 have been deleted;

2) an HCV non-core protein, and

3) at least one anti-HCV core monoclonal antibody that binds to HCV core antigen and does not bind with the HCV core peptide described in A) 1); and

B) a labeled anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind to the HCV core peptide described in A) 1) and does not bind to the same epitope as the anti-HCV core monoclonal antibody described in A) 3).

In another embodiment, there is provided an isolated hepatitis C virus (HCV) core peptide comprising the amino acid sequence of SEQ ID NO.: 19, wherein amino

acids 1-8 MSTNPKPQ of SEQ ID NO:19 have been deleted and amino acids 31-33 VGG of SEQ ID NO:19 have been deleted.

Detailed Description of the Invention

One object of the invention is to develop peptide sequences that can detect anti-HCV antibodies in the presence of anti-core monoclonal antibodies to detect HCV core antigen. One use therefore of these modified core antigens will be to use them in an anti-HCV/HCV core assay, or "combination assay".

For this purpose, two or more monoclonal antibodies from Table 4 can be co-coated with one or more modified HCV core proteins on a solid phase thereby enabling the solid phase to capture anti-HCV core antibodies and core antigen. The modification is accomplished by removing the epitope for the antibody being used for detection or capture of HCV core. The removal of epitopes can be achieved by means known in the art such as deleting parts of the core sequence or altering amino acids in

the epitope sequence. This can be achieved, for example, by synthesizing these peptides by chemical synthesis using commercially available peptide synthesizers or by modifying recombinant clones expressing HCV core protein. The recombinant sequences can be modified by primer dependent single or multiple site mutagenesis or primer dependent deletions. (B. Tao and K.C. Lee, PCR Technology Current Innovations,1994 by CRC Press, Inc., Chapter 10, Mutagenesis by PCR).

Another object of the invention is to identify immunodominant regions of HCV core protein.

Another object of the present invention is to determine the pattern of reactivity to core peptides among HCV infected individuals presenting anti-core antibodies.

The peptides of the invention were generated by maintaining the highly reactive portions of the core protein and making modifications to the remaining parts of the sequence such that the peptide would not be detected by an antibody used to detect core protein in an assay.

More particularly, there is described a combination for the simultaneous detection of hepatitis C virus (HCV) antibody and HCV antigen comprising:

A) a solid phase coated with

1) an HCV core peptide, comprising the amino acid sequence of SEQ ID NO:19, wherein at least two of the following amino acid segments have one or more amino acids deleted,

i) MSTNPKPQRK (residues 1-10);

ii) IVGGVYLL (residues 30-37);

iii) RLGVRATR (residues 43-50); and

iv) AQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTD PRRRSRNLGKVIDTLTCGF (residues 77-130);

2) an HCV non-core protein, and

3) at least one anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind with the HCV core peptide described in A) 1); and

B) a labeled anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind to the HCV core peptide described in A) 1) and does not bind to the same epitope as the anti-HCV monoclonal antibody described in A) 3).

In a preferred embodiment, the peptide would be modified by either substitution of amino acids or deletion of amino acids in the regions underlined in Table 3. The remainder of the sequence shown in Table 3 should not be altered.

In another preferred embodiment the peptides would be used in a combination assay. That is one that is capable of detecting both HCV antigens and antibodies simultaneously.

Table 3

HCV core protein sequence:

SEQ ID NO.: 19

MSTNPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRK TSERSQPRGRRQPIPKARRPEGRSWAQPGYPWPLYGNEGCGWAGWLLSPRGSRPSW GPTDPRRRSRNLGKVIDTLTCGF

The effectiveness and advantages of the invention are further illustrated by the following examples.

Example 1

Synthetic Peptides

Synthetic peptides covering the entire sequence of HCV core protein sequence were used. Eighteen overlapping peptides shown in Table 1 were chemically synthesized by solid phase peptide synthesis using a peptide synthesizer. All of the peptides were synthesized with a C-terminal cysteine amide residue. Peptides were cleaved form the resin and purified by reverse phase liquid chromatography. Purity of each of these peptides, based on reverse phase HPLC analysis was greater that ninety-five (95%). The sequence of each peptide was confirmed by amino acid analysis of the acid hydrolysate of the peptide. The structural identities of the peptides were confirmed by mass spectrometry. All of the peptides had the molecular weight expected.

Example 2

Synthetic peptides were coated onto Immulon™ 2 microwells (made by Dyanatech) in 50 mM borate buffer at a concentration of 1 ug/ml. To each microwell, 200 ul of peptide solution was added and the microwells were incubated at 25 degrees Celsius for 16-20 hours. The microwells were aspirated and washed once with phosphate

buffer saline (PBS)containing TWEEN 20™ to remove any unbound peptide. The microwells were then post coated with 300 ul of PBS containing one percent (1%)bovine serum albumin (BSA) and three percent (3%) sucrose to block all of the available protein binding sites. After 2-4 hours, the plates were aspirated, turbo dried and stored in sealed pouches at 2-8 degrees Celsius.

Example 3

Synthetic Peptide ELISA

The sample, 10 ul in 200 ul of diluent, suspected of being infected with HCV was added into the peptide coated microwell. After incubation for approximately one hour the microwells were washed. To the washed microwells anti-human IgG labeled with horse radish peroxidase was added. After an incubation for thirty minutes the microwells were washed and a solution of ORTHO phenylenediamine buffer and hydrogen peroxide was added to each well. After approximately 30 minutes sulfuric acid were added to each well to stop the reaction. An orange or yellow color indicated the presence of anti-HCV antibodies in the sample.

Table 4

Monoclonal Antibodies

The table below identifies 15 antibodies. The antibodies were screened at every stage of antibody development with microtitrewell plates coated with immunogen. The immunogen used to immunize the mice that produced each strain of monoclonal antibody is identified as one of the following: peptide ODS 243, a large peptide further defined in Example 1; "FLC" means full length core antigen, defined in Example 1; or KLH conjugated core peptide #8, a short peptide, further defined herein. The specificity of each to a numbered peptide is shown and the amino acid sequences of each numbered peptide is identified herein. Furthermore, the epitope to which the antibody specifically binds is included in the last column, defined by the amino acids that encode for the epitope.

ATCC #	**AG-FUSION #, clone**	**IMMUNOGEN**	ISOTYPE	**SPECIFICITY**	**AA**
PTA-3811	ODS243,7B4F11	Peptide ODS 243	IgG 2b	HCV core peptide #8	77-91
PTA-3803	ODS243,1E3D12	Peptide ODS 243	IgG2a	HCV core peptide #9	86-100
PTA-3802	ODS243,7C12C4	Peptide ODS 243	IgG2b	HCV core peptide #8	77-91
PTA-3813	core#3, 2A11C6	FLC	IgG1	HCV core peptide# 11	106-120
PTA-3809	Core#12, 1B7A1	FLC	IgG1	HCV core peptide# 3	29-43
PTA-3805	Core#13, 5A12G12	FLC	IgG1	HCV core peptide# 4	39-53
PTA-3812	Core#13, 4H7E7	FLC	IgG1	HCV core peptide# 5	48-62
PTA-3806	Core#13,12F4A 11	FLC	IgG1	HCV core peptide# 6	58-72
PTA-3804	Core#13, 14D12A12	FLC	IgG1	HCV core peptide# 7	67-81
PTA-3807	c22-8#4, 6D8E8	KLH Conjugated core peptide #8	IgG1	HCV core peptide# 8	77-91
PTA-3800	Core#12, 4G10G6	FLC	IgG2b	HCV core peptide# 10	96-110
PTA-3801	Core#13, 6E7E1	FLC	IgG2a	HCV core peptide# 11	106-120
PTA-3810	Core#13, 11D12A6	FLC	IgG2b	HCV core peptide# 11	106-120
PTA-3808	Core#13, 14B7C3	FLC	IgG3	HCV core peptide# 11	106-120
PTA-3799	Core#12, 4A6H3	FLC	IgG1	HCV core peptide# 16	156-170

Example 4

Use of modified core peptides in an anti-HCV ELISA

A peptide consisting of HCV core immunodominant region amino acids 1-43 was chemically synthesized. Another peptide with certain deletions in this region, namely, amino acids 1-8 and 31-33 were deleted, was also synthesized. These peptides were used to test 40 chronic HCV patients' serum samples for anti-HCV antibody status. The results indicated that 39 of the 40 patients were not affected by deletion of these sequences. The deletion peptides can be used in combination with anti-HCV monoclonal antibodies, such as the ones shown in Table 4, in a combination assay. Peptides with deletions of amino acids 31-33 can be used with any of the monoclonal antibodies shown in Table 4.

Table 5

Peptide/Specimen ID	265-2	266-2	271-2	272-2
11	2.500	2.500	2.500	2.500
15	2.500	2.500	2.500	2.500
18	2.500	2.500	2.500	2.500
20	2.500	2.500	2.500	2.500
21	2.500	2.500	2.500	2.500
27	2.500	2.500	2.500	2.500
30	2.500	2.500	2.500	2.500

Table 5				
Peptide/Specimen ID	**265-2**	**266-2**	**271-2**	**272-2**
35	**0.762**	**0.911**	**0.507**	**0.611**
36	**2.500**	**2.500**	**2.500**	**2.500**
37	**2.500**	**2.500**	**2.500**	**2.500**
39	**2.500**	**2.500**	**2.500**	**2.500**
40	**2.500**	**2.500**	**2.500**	**2.500**
42	**2.500**	**2.500**	**2.500**	**2.500**
43	**1.621**	**1.255**	**1.382**	**1.443**
44	**2.500**	**2.500**	**2.500**	**2.500**
45	**2.500**	**2.500**	**2.500**	**2.500**
46	**2.003**	**1.863**	**1.058**	**1.829**
47	**2.500**	**2.500**	**2.500**	**2.500**
48	**2.500**	**2.500**	**2.500**	**2.500**
49	**2.500**	**2.500**	**2.500**	**2.500**
50	**2.500**	**2.500**	**2.500**	**2.500**
52	**2.500**	**2.500**	**2.500**	**2.500**
53	**2.500**	**2.500**	**2.500**	**2.500**
54	**2.500**	**2.500**	**2.500**	**2.500**
55	**2.500**	**2.500**	**2.500**	**2.500**
58	**2.500**	**2.500**	**2.500**	**2.500**
59	**2.500**	**2.500**	**2.500**	**2.500**
60	**2.500**	**2.500**	**2.500**	**2.500**
62	**2.500**	**2.500**	**2.500**	**2.500**
65	0.056	0.045	**2.237**	**1.704**
66	**2.500**	**2.500**	**2.500**	**2.500**
67	**2.500**	**2.500**	**2.500**	**2.500**
68	**2.500**	**2.500**	**2.500**	**2.500**
70	**2.500**	**2.500**	**2.500**	**2.500**
73	**2.500**	**2.500**	**2.500**	**2.500**
75	**2.500**	**2.500**	**2.500**	**2.500**
78	**2.500**	**2.500**	**2.500**	**2.500**
80	**2.500**	**2.500**	**2.500**	**2.500**
84	**2.500**	**2.500**	**2.170**	**2.500**
90	**2.500**	**2.500**	**2.500**	**2.500**
Peptide 265 HCV Polyprotein Amino Acid Sequence 10-43				
Peptide 266 HCV Polyprotein Amino Acid Sequence 8-43				
Peptide 271 HCV polyprotein Amino Acid Sequence 1-43 with AA 31,32 and 33 deleted				
Peptide 272 HCV Polyprotein Amino Acid Sequence 1-43 Consensus sequence				

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ortho-Clinical Diagnostics Inc, .

(ii) TITLE OF INVENTION: HCV Core Protein Sequences

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Suite 1600
(B) STREET: McGill College Avenue 1981
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(F) ZIP: H3A 2Y3

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE: 11-NOV-2001

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(B) FILING DATE: 10-OCT-2002

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ser Thr Asn Pro Lys Pro Gln Lys Lys Asn Lys Arg Asn Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Asn Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Pro	Arg	Gly	Arg	Arg	Gln	Pro	Ile	Pro	Lys	Ala	Arg	Arg	Pro	Glu
1				5				10						15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys	Ala	Arg	Arg	Pro	Glu	Gly	Arg	Thr	Trp	Ala	Gln	Pro	Gly	Tyr
1				5				10						15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala	Gln	Pro	Gly	Tyr	Pro	Trp	Pro	Leu	Tyr	Gly	Asn	Glu	Gly	Cys
1				5				10						15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Tyr	Gly	Asn	Glu	Gly	Cys	Gly	Trp	Ala	Gly	Trp	Leu	Leu	Ser	Pro
1				5				10						15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Trp	Leu	Leu	Ser	Pro	Arg	Gly	Ser	Arg	Pro	Ser	Trp	Gly	Pro	Thr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg Leu Asn Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ser Arg Leu Asn Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Thr Cys Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Arg Val Leu Glu Asp Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis C Virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
1 5 10 15

Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
20 25 30

Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
35 40 45

Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
50 55 60

Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Ser Trp Ala Gln Pro Gly
65 70 75 80

Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp
85 90 95

Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
100 105 110

Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
115 120 125

Gly Phe
130

CLAIMS

1. A combination for the simultaneous detection of hepatitis C virus (HCV) antibody and HCV antigen comprising:

A) a solid phase coated with

1) an HCV core peptide, comprising the amino acid sequence of SEQ ID NO:19, wherein at least two of the following amino acid segments have one or more amino acids deleted,

i) MSTNPKPQRK (residues 1-10);

ii) IVGGVYLL (residues 30-37);

iii) RLGVRATR (residues 43-50); and

iv) AQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTD PRRRSRNLGKVIDTLTCGF (residues 77-130);

2) an HCV non-core protein, and

3) at least one anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind with the HCV core peptide described in A) 1); and

B) a labeled anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind to the HCV core peptide described in A) 1) and does not bind to the same epitope as the anti-HCV core monoclonal antibody described in A) 3).

2. The combination according to claim 1 wherein the HCV non-core protein is NS5.

3. The combination according to claim 1 wherein the labeled anti-HCV core monoclonal antibody is labeled with an enzyme, a fluorophore, or a radioactive label.

4. The combination according to claim 1 wherein the solid phase is a microtitre well, polystyrene bead, magnetic bead or membrane.

5. An isolated HCV core peptide comprising the amino acid sequence of SEQ ID NO.: 19, wherein at least two of the following segments of amino acids have one or more amino acids deleted,

i) MSTNPKPQRK (residues 1-10);

ii) IVGGVYLL (residues 30-37);

iii) RLGVRATR (residues 43-50); and

iv) AQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLGKV IDTLTCGF (residues 77-130),

and wherein amino acids 11-29, 38-42, and 51-76 are not deleted or altered.

6. The isolated HCV core peptide claimed in claim 5 wherein amino acids MSTNPKPQ have been deleted from segment i) and amino acids VGG have been deleted from segment ii).

7. A combination for the simultaneous detection of hepatitis C virus (HCV) antibody and HCV antigen comprising:

A) a solid phase coated with

1) an HCV core peptide, comprising the amino acid sequence of SEQ ID NO:19, wherein amino acids 1-8 MSTNPKPQ of SEQ ID NO:19 have been deleted and amino acids 31-33 VGG of SEQ ID NO:19 have been deleted;

2) an HCV non-core protein, and

3) at least one anti-HCV core monoclonal antibody that binds to HCV core antigen and does not bind with the HCV core peptide described in A) 1); and

B) a labeled anti-HCV core monoclonal antibody that binds to HCV core antigen present in a sample from an HCV infected individual and does not bind to the HCV core peptide described in A) 1) and does not bind to the same epitope as the anti-HCV core monoclonal antibody described in A) 3).

8. An isolated hepatitis C virus (HCV) core peptide comprising the amino acid sequence of SEQ ID NO.: 19, wherein amino acids 1-8 MSTNPKPQ of SEQ ID NO:19 have been deleted and amino acids 31-33 VGG of SEQ ID NO:19 have been deleted.