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**A Hepatitis C Antigen - Antibody Combination Assay
for the Early Detection of HCV Infection**

Background of the Invention

5 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).

10 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 spliced into at least
10 functional proteins. Clearly, the ability to test
15 the blood supply for HCV is of great importance.
Furthermore, the earlier the stage of HCV one can
detect, the better.

 Therefore, we have developed an ELISA based assay
for the detection of HCV infection. This new assay can
20 detect HCV infection earlier than the currently used
assays for the screening of blood for HCV infection.
Current assays are based on the detection of anti-HCV
antibodies in infected blood by capturing these

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antibodies by HCV protein sequences represented by recombinant proteins or peptides.

For example, the Ortho HCV 3.0 ELISA uses multiple antigens to detect anti-HCV antibodies very early in seroconversion. However, there is a significant window period in viremic individuals before they develop anti-HCV antibodies. This window period can be as long as 60 days. During this period the individual, though highly infective, will go undetected by current HCV screening assays.

Summary of the Invention

One object of the invention is to provide a diagnostic test that can detect HCV infection during the window period described above by capturing both HCV core antigen and antibodies. We describe herein a method for detecting HCV infection earlier than the currently used antibodies assays. This has been accomplished by providing a test that detects HCV core antigen in addition to the anti-HCV antibodies.

Another object of the invention is to provide a method for determining the presence of HCV in a sample,

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comprising, contacting the sample with HCV antigens and anti -HCV core antibodies attached to a solid phase, adding a polyoxyethylene ether to said sample, detecting captured antigens and antibodies by adding labeled anti-
5 human IgG and labeled anti-HCV core antibodies and detecting the signal as an indication of the presence of HCV infection.

Another object of the invention is to provide certain detergents that are needed to release the HCV
10 core antigen from the virus by disrupting the envelope protein and/or the lipid layer.

Detailed Description of the Invention

It is preferable in a combination assay for the detergents to release the core antigen from the virus
15 and yet not have a negative impact on the ability of the HCV recombinant proteins to capture anti-HCV antibodies.

In a preferred embodiment of the invention, detergents from the polyoxyethylene ethers class are used for this purpose. Commonly available detergents in this class
20 are: BRIJ 30, BRIJ 35, 56, 58, 92, 96, 98, 700 and MYRJ 52, 59, 53, 45. These detergents help in releasing the HCV

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core antigen from the virus but the presence of these detergents do not negatively impact the anti-HCV assay. Certain detergents can effect the antibody detection by either affecting the recombinant antigens coated on the solid phase or inactivating the anti-HCV antibodies in the sample. Some of the detergents like N-Lauryl Sarcosine used in the detection of HCV core assay destroy the ability of HCV recombinant proteins c22-3, c200 and NS5 to detect anti HCV antibodies in Ortho HCV 3.0 ELISA.

As used herein a "sample", refers to any substance which may contain the analyte of interest. A sample can be biological fluid, such as whole blood or whole blood components including red blood cells, white blood cells, platelets, serum and plasma, ascites, urine, cerebrospinal fluid, and other constituents of the body which may contain the analyte of interest.

Examples:

The following examples demonstrate the advantages and utility of the invention by describing a list of detergents evaluated and the results obtained by using

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the above described assay. One skilled in the art would recognize that the detection of HCV antibody and antigen can be done separately or simultaneously. These examples are meant to illustrate, but not limit, the spirit and scope of the invention.

Example 1: An Antigen-antibody combination assay.

HCV antigens c200-3, NS-5 and a modified core antigen C22KSÑ 47,48 or c22KSR47L along with two anti-core monoclonal antibodies (anti core murine monoclonals Pep10,12) were coated onto microwells in phosphate buffer. Antigens were modified by modifying the DNA clones coding for the recombinant proteins c22-3 containing the HCV1 sequence. These procedures involve site directed mutagenesis using synthetic oligonucleotides (Sambrook, Fritsh and Maniatis in Molecular Cloning, A laboratory manual, second edition, Cold Spring Harbor Press, Chapter 15, 1989). After overnight incubation the buffer containing the coating proteins was removed and the microwells washed with phosphate buffered saline containing a detergent Tween 20. The antigen/antibody coated microwells were then

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treated with BSA/sucrose solution to block all of the protein binding sites on the microwells. After 2-24 hours the BSA/sucrose solution was removed and the microwells air dried and stored under a desiccant.

5 Example 2: 100mL of sample to be tested was diluted into 100 mL of PBS solution containing bovine serum albumin, superoxide dismutase, yeast extract and 1% Brij 58 or Brij 35 or a mixture of both. The diluted specimens were pipetted into HCV antigen/monoclonal antibodies coated microwells (as described in Example 10 1). These microwells were incubated at 37°C for 90 minutes. The microwells were then washed 5 times with PBS containing 0.5% Tween 20. A 200mL solution of a murine anti-human IgG labeled with horseradish 15 peroxidase (HRP) and an anti-HCV core monoclonal antibody (Anti Pep4) labeled with HRP was added. After an incubation of 30 minutes the microwells were washed 5 times with PBS Tween 20. A solution of orthophenylenediamine and hydrogen peroxide was added to 20 each well. After incubation in the dark for 30 minutes the reaction was stopped by adding 50 microliters of 4N

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sulfuric acid. The plates were read at 495nm. An orange color in either well indicates that the specimen being tested is infected with HCV.

Example 3 Effects of polyoxyethylene ethers on HCV

5 antibody or antigen assays

Effect of the addition of various polyoxyethylene ethers on the HCV 3.0 anti-HCV assay or HCV core antigen ELISA assay is shown below in Tables 1A and 1B. In the HCV core antigen ELISA assay N-Lauryl sarcosine used in the
10 commercial HCV core antigen was replaced by different concentrations of detergents in column one. In the HCV 3.0 anti-HCV assay the detergents were added to the specimen diluent used in the HCV 3.0 assay.

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Table 1A.

Detergent Tested			HCV 3.0 Ab ELISA					
			Base Matrix			HCV Antibody Reactive		
			0.25%	0.125%	0.06%	0.25%	0.125%	0.06%
			0.125%	0.06%	0.03%	0.125%	0.06%	0.03%
SYN.								
polyoxyethylene	2 oleyl ether	Brij 92	0.056	0.035	0.041	1.543	1.432	1.405
polyoxyethylene	8 stearate	Myrj 45	0.019	0.024	0.025	1.208	1.286	1.313
polyoxyethylene	5 lauryl ether		0.033	0.046	0.058	1.168	1.274	1.351
polyoxyethylene	50 stearate	Myrj 53	0.044	0.069	0.083	1.368	1.361	1.262
polyoxyethylene	8 lauryl ether		0.064	0.070	0.059	1.223	1.219	1.277
polyoxyethylene	9 lauryl ether		0.057	0.055	0.061	1.312	1.260	1.225
polyoxyethylene	10 lauryl ether		0.049	0.059	0.064	1.192	1.069	1.161
polyoxyethylene	10 cetyl ether	Brij 56	0.053	0.093	0.059	0.994	1.212	1.209
polyoxyethylene	10 oleyl ether	Brij 97	0.031	0.056	0.072	1.316	1.341	1.534
polyoxyethylene	100 stearate	Myrj 59	0.047	0.050	0.045	1.751	1.615	1.418
polyoxyethylene	20 cetyl ether	Brij 58	0.058	0.046	0.062	1.568	1.507	1.488
polyoxyethylene	40 stearate	Myrj 52	0.048	0.063	0.067	1.502	1.308	1.194
polyoxyethylene	100 stearyl ether	Brij 700	0.043	0.045	0.052	1.600	1.482	1.503
polyoxyethylene	20 oleyl ether	Brij 98	0.053	0.044	0.050	1.632	1.542	1.493
polyoxyethylene	23 lauryl ether	Brij 35	0.067	0.055	0.065	1.492	1.415	1.473
polyoxyethylene	7 lauryl ether		0.065	0.105	0.100	1.151	1.458	1.497
polyoxyethylene	4 lauryl ether	Brij 30	0.093	0.058	0.185	0.867	1.135	1.190
polyoxyethylene	3 lauryl ether		0.035	0.042	0.075	0.995	0.927	1.156
Pluronic F-127			0.025	0.030	0.035	1.521	1.327	1.271
Control			0.075 (3.0 SD)			1.358 (3.0 SD)		

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Table 1B.

Detergent Tested			HCV Antigen Prototype					
			Base Matrix			HCV Antigen Reactive		
			0.25%	0.125%	0.06%	0.25%	0.125%	0.06%
			0.125%	0.06%	0.03%	0.125%	0.06%	0.03%
	SYN.							
polyoxyethylene 2 oleyl ether	Brij 92		0.010	0.010	0.032	0.609	0.847	0.502
polyoxyethylene 8 stearate	Myrj 45		0.028	0.021	0.022	0.880	0.509	0.433
polyoxyethylene 5 lauryl ether			0.002	0.004	0.007	0.195	0.243	0.265
polyoxyethylene 50 stearate	Myrj 53		0.014	0.040	0.006	0.573	0.536	0.659
polyoxyethylene 8 lauryl ether			0.009	0.010	0.014	0.326	0.384	0.343
polyoxyethylene 9 lauryl ether			0.008	0.008	0.005	0.360	0.379	0.212
polyoxyethylene 10 lauryl ether			0.010	0.013	0.020	0.495	0.556	0.344
polyoxyethylene 10 cetyl ether	Brij 56		0.004	0.001	0.016	0.445	0.298	0.390
polyoxyethylene 10 oleyl ether	Brij 97		0.004	0.006	0.021	0.371	0.419	0.479
polyoxyethylene 100 stearate	Myrj 59		0.019	0.021	0.023	0.429	0.320	0.369
polyoxyethylene 20 cetyl ether	Brij 58		0.008	0.011	0.028	0.546	0.898	0.564
polyoxyethylene 40 stearate	Myrj 52		0.009	0.005	0.006	0.579	0.412	0.661
polyoxyethylene 100 stearyl ether	Brij 700		0.016	0.023	0.019	0.294	0.307	0.319
polyoxyethylene 20 oleyl ether	Brij 98		0.008	0.011	0.032	0.557	0.718	0.539
polyoxyethylene 23 lauryl ether	Brij 35		0.022	0.028	0.029	0.722	0.494	0.309
polyoxyethylene 7 lauryl ether			0.009	0.009	0.016	0.228	0.289	0.309
polyoxyethylene 4 lauryl ether	Brij 30		-0.001	0.001	0.008	0.194	0.158	0.212
polyoxyethylene 3 lauryl ether			0.004	0.005	0.013	0.106	0.168	0.162
Pluronic F-127			0.013	0.013	0.012	0.324	0.285	0.268
Control			0.001 (nLs)			1.109 (nLs)		

Example 4 Detection of HCV core antigen and/or anti-HCV antibodies.

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Detection of HCV core antigen or anti-HCV antibodies on plates coated with anti-core monoclonal antibodies c11-3 and c11-7 and HCV antigens c200-3, NS5 and a modified core antigen. (SOD fused protein containing core sequences aa10-99 with aa 47 and 48

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deleted (c22KS(∇47-48)). The specimens used were 4 sequential bleeds from a commercially available seroconversion panel. Core antigen was detected using c11-4 monoclonal antibody labeled with HRP, anti-HCV antibodies were detected by anti-human IgG labeled with HRP. By using the two antibodies together a cumulative signal was produced, as shown in the column labeled combo in Table 2.

Table 2

SAMPLE	ANTI IgG	ANTI HCV CORE	COMBO
NEG MEAN*	0.045	0.036	0.094
BCP 6215 1	0.007	0.135	0.193
BCP 6215 2	0.009	0.157	0.191
BCP 6215 3	0.014	0.231	0.270
BCP 6215 4	0.494	0.197	0.614

*signal obtained by using HCV negative samples

Example 5 Detection of HCV core antigen and/or anti-HCV antibodies.

Detection of HCV core antigen and/or anti-HCV antibodies on plates coated with anti-core monoclonal antibodies c11-3 and c11-7 and HCV antigens c200-3, NS5 and a modified core antigen. (SOD fused protein

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containing core sequences aa10-99 with arginine at position 47 replaced with a leucine (c22KS (R47L)). The specimens used were 4 sequential bleeds from a commercially available seroconversion panel. Core antigen was detected using c11-4 monoclonal antibody labeled with HRP, anti-HCV antibodies were detected by anti-human IgG labeled with HRP. In the combo assay the two detecting antibodies, anti core monoclonal and anti-human IgG monoclonal antibody labeled with HRP were used as a mixture.

Table 3

SAMPLE	ANTI IgG	ANTI HCV CORE	COMBO
NEG MEAN	0.045	0.036	0.094
BCP 6215 1	0.007	0.135	0.193
BCP 6215 2	0.009	0.157	0.191
BCP 6215 3	0.014	0.231	0.270
BCP 6215 4	0.494	0.197	0.614

Example 6 Anti-IgG Comparison.

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A comparison of anti-HCV reactivity of seroconverter panel in microwells coated with two different HCV core proteins is shown in Table 4.

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Table 4

SAMPLE	SOD / c22KS (Δ 47-48)	SOD / c22KS (R47L)
NEG MEAN	0.045	0.076
BCP 6215 1	0.007	0.015
BCP 6215 2	0.009	0.021
BCP 6215 3	0.014	0.028
BCP 6215 4	0.494	0.498

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Example 7 Comparison of core antigen detection of seroconverter panel in microwells coated with two different core antigens is shown in Table 5.

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Table 5

Anti-HCV Core Comparison

SAMPLE	SOD / c22KS (Δ 47-48)	SOD / c22KS (R47L)
NEG MEAN	0.036	0.047
BCP 6215 1	0.135	0.224
BCP 6215 2	0.157	0.189
BCP 6215 3	0.231	0.304
BCP 6215 4	0.197	0.189

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Example 8 Comparison of combo reactivity of seroconverter panel in microwells coated with two different core antigens is shown in Table 6.

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Table 6

HCV Combo Comparison

SAMPLE	SOD / c22KS (Δ 47-48)	SOD / c22KS (R47L)
NEG MEAN	0.094	0.144
BCP 6215 1	0.193	0.247
BCP 6215 2	0.191	0.226
BCP 6215 3	0.270	0.328
BCP 6215 4	0.614	0.658

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We claim:

1. A method for determining the presence of HCV in a sample, comprising:

5 contacting the sample with HCV antigens and anti -
HCV core antibodies attached to a solid phase,
adding a polyoxyethylene ether selected from the
group consisting of polyoxyethylene 2 oleyl ether,
polyoxyethylene 8 stearate, polyoxyethylene 5
10 lauryl ether, polyoxyethylene 50 stearate,
polyoxyethylene 8 lauryl ether, polyoxyethylene 9
lauryl ether, polyoxyethylene 10 lauryl ether,
polyoxyethylene 10 cetyl ether, polyoxyethylene 10
oleyl ether, polyoxyethylene 100 stearate,
15 polyoxyethylene 20 cetyl ether, polyoxyethylene 40
stearate, polyoxyethylene 100 stearyl ether,
polyoxyethylene 20 oleyl ether, polyoxyethylene 23
lauryl ether, polyoxyethylene 7 lauryl ether,
polyoxyethylene 4 lauryl ether, polyoxyethylene 3
20 lauryl ether and Pluronic F-127 to said sample,
detecting captured antigens and antibodies by
adding labeled anti-human IgG and labeled anti-HCV
core antibodies and detecting the signal as an
indication of the presence of HCV infection.