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(54) **METHODS FOR THE SIMULTANEOUS DETECTION OF HCV ANTIGENS AND HCV ANTIBODIES**

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C12Q 1/70 (2006.01)
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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,322,769	A	6/1994	Bolling et al.	
5,486,479	A *	1/1996	Ito et al.	436/533
6,486,479	B1	6/1996	Ito et al.	
5,627,026	A *	5/1997	O'Connor et al.	435/5
5,705,330	A	1/1998	Shah et al.	
5,753,430	A	5/1998	Mehta et al.	
6,018,047	A	1/2000	Zomer et al.	
6,172,189	B1	1/2001	Devare et al.	
6,312,889	B1	11/2001	Houghton et al.	
6,383,740	B1 *	5/2002	Collins	435/5
6,623,921	B1 *	9/2003	Aoyagi et al.	435/5
2002/0173493	A1	11/2002	Aoyagi et al.	
2002/0192639	A1 *	12/2002	Chien et al.	
2003/0049608	A1 *	3/2003	Bahl et al.	

FOREIGN PATENT DOCUMENTS

GB	2051357	1/1980	
GB	2313666	12/1997	
WO	97/01758	1/1997	
WO	99/06836	11/1999	
WO	WO 00/07023	* 2/2000	
WO	01/96875 A2	12/2001	

OTHER PUBLICATIONS

Masalova et al., Detection of Hepatitis C Virus Core Protein Circulating Within Different Virus Particle Populations, Journal of Medical Virology 55:1-6, 1998.*

Jolivet-Reynaud et al., HCV Core Immunodominant Region Analysis Using Mouse Monoclonal Antibodies and Human Sera: Characterization of Major Epitopes Useful for Antigen Detection. Journal of Medical Virology 56:300-309, 1998.*

Kashiwakuma et al., Detection of hepatitis C virus specific core protein in serum of patients by a sensitive fluorescence enzyme immunoassay (FEIA). Journal of Immunological Methods 190:79-89, 1996.*

Major, et al., *Hepatology,* 25:1527 (1997).

Muerhoff A.S., et al., *J. Virol,* 69;5621 (1995).

Choo, et al., *Proc. Natl. Acad. Sci. USA,* 88:2451 (1991).

Grakoui, et al., *J. Virol,* 67:1385 (1993.

Busch, et al., *Transfusion,* 40:143 (2000).

Choo, et al., *Science,* 244:359 (1989).

Kuo, et al., *Science,* 244:362 (1989).

Mimms, et al., *Lancet,* 336:1590 (1990).

Bresters, et al., *Vox Sang,* 62:213 (1992).

Kleinman, et al., *Transfusion,* 32:805 (1992).

Hino K., *Intervirology,* 37:77 (1994).

Alter, et al., *N. Engl. J. Med.,* 327:1899 (1992).

Lee, et al., *Transfusion,* 35:845 (1995).

Courouce, et al., *Transfusion,* 34:790-795 (1994).

Courouce, et al., *Lancet,* 343:853 (1994).

Aoyagi, et al., *J. Clin Microbiol.,* 37:1802 (1999).

Peterson, et al., *Vox Sang,* 78:80 (2000).

Dawson, et al., *Transfusion,* SD161, 40 (2000).

Tanaka, et al., *Hepatology,* 32:388 (2000).

Tanaka, et al., *J. Hepatology,* 23:742 (1995).

Kohler and Milstein, *Nature,* 256:494 (1975).

Frank Quinn, *The Immunoassay Handbook, Second Edition,* pp. 363-367 (2001).

G. Reyes, et al., *Mol. Cell. Probes,* 5:473:481 (1991).

* cited by examiner

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(57) **ABSTRACT**

The subject invention relates to methods for the simultaneous detection of Hepatitis C Virus (HCV) antigens as well as antibodies produced in response to HCV antigens. Furthermore, the subject invention allows one to detect antigens in the early, acute stage of infection, even prior to the development of antibodies, thereby allowing for early detection of infected blood and blood products, thus improving the safety of the blood supply.

2 Claims, 3 Drawing Sheets

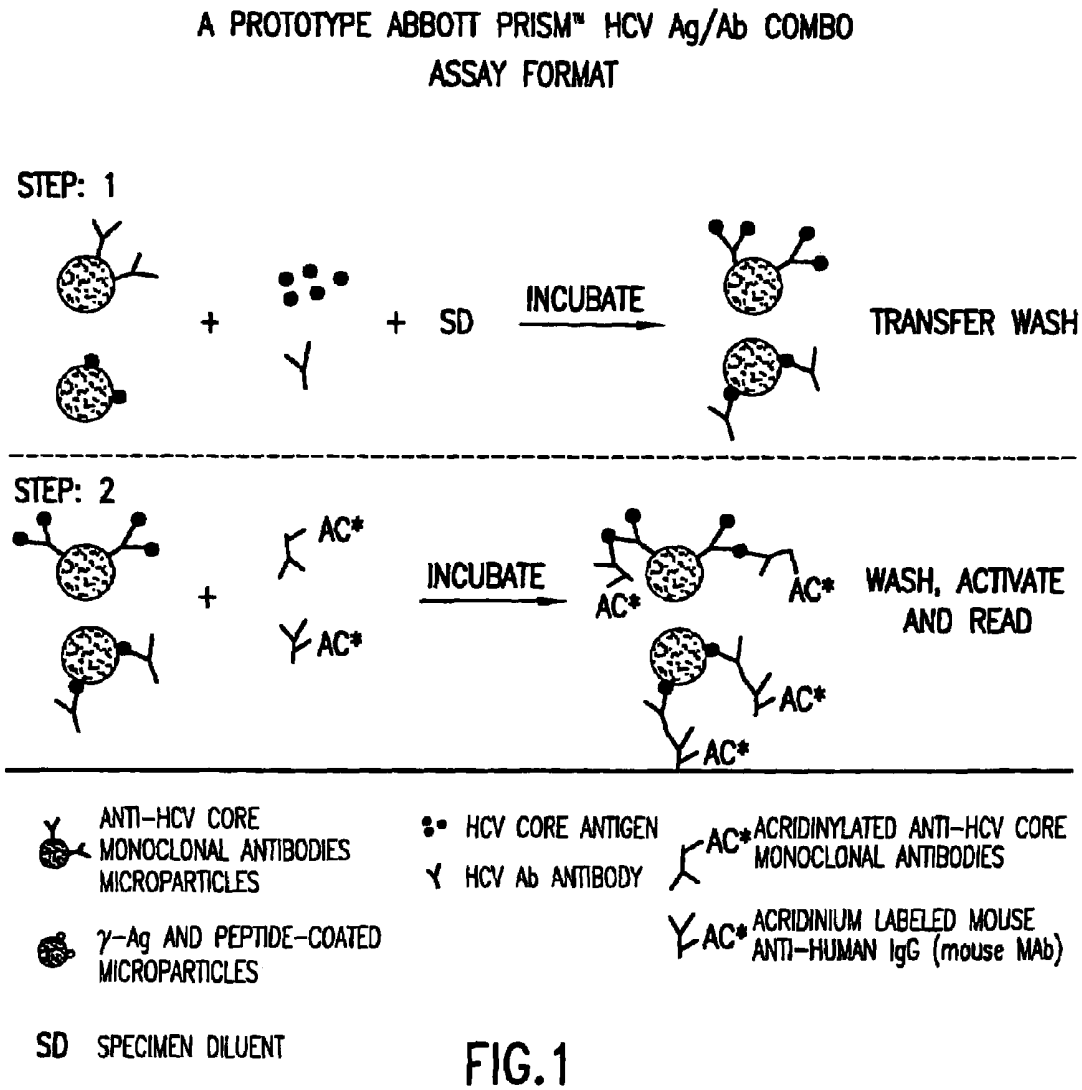
A PROTOTYPE ABBOTT PRISM™ HCV Ag/Ab COMBO
ASSAY FORMAT
STEP: 1
+
+ SD
INCUBATE
TRANSFER WASH
STEP: 2
AC*
AC*
+
INCUBATE
AC*
AC*
AC*
AC*
WASH, ACTIVATE
AND READ
ANTI-HCV CORE
MONOCLONAL ANTIBODIES
MICROPARTICLES
γ-Ag AND PEPTIDE-COATED
MICROPARTICLES
SD SPECIMEN DILUENT
HCV CORE ANTIGEN
HCV Ab ANTIBODY
AC* ACRIDINYLATED ANTI-HCV CORE
MONOCLONAL ANTIBODIES
AC* ACRIDINIUM LABELED MOUSE
ANTI-HUMAN IgG (mouse MAb)

FIG.1

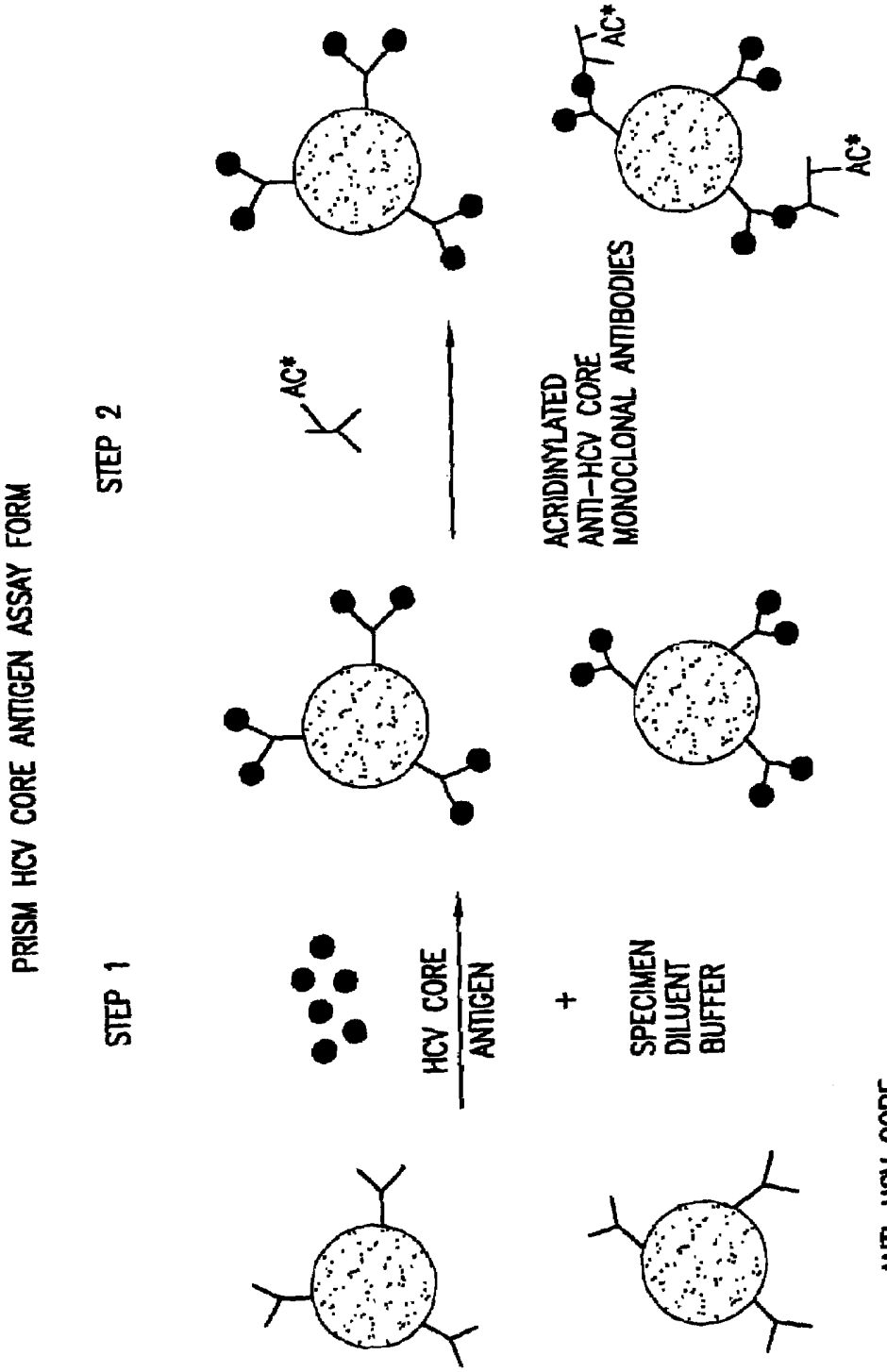
PRISM HCV CORE ANTIGEN ASSAY FORM
STEP 1
HCV CORE ANTIGEN
+
SPECIMEN DILUENT BUFFER
ANTI-HCV CORE MONOCLONAL ANTIBODIES
STEP 2
AC*
ACRIDINYLATED ANTI-HCV CORE MONOCLONAL ANTIBODIES
AC*
AC*

FIG.2

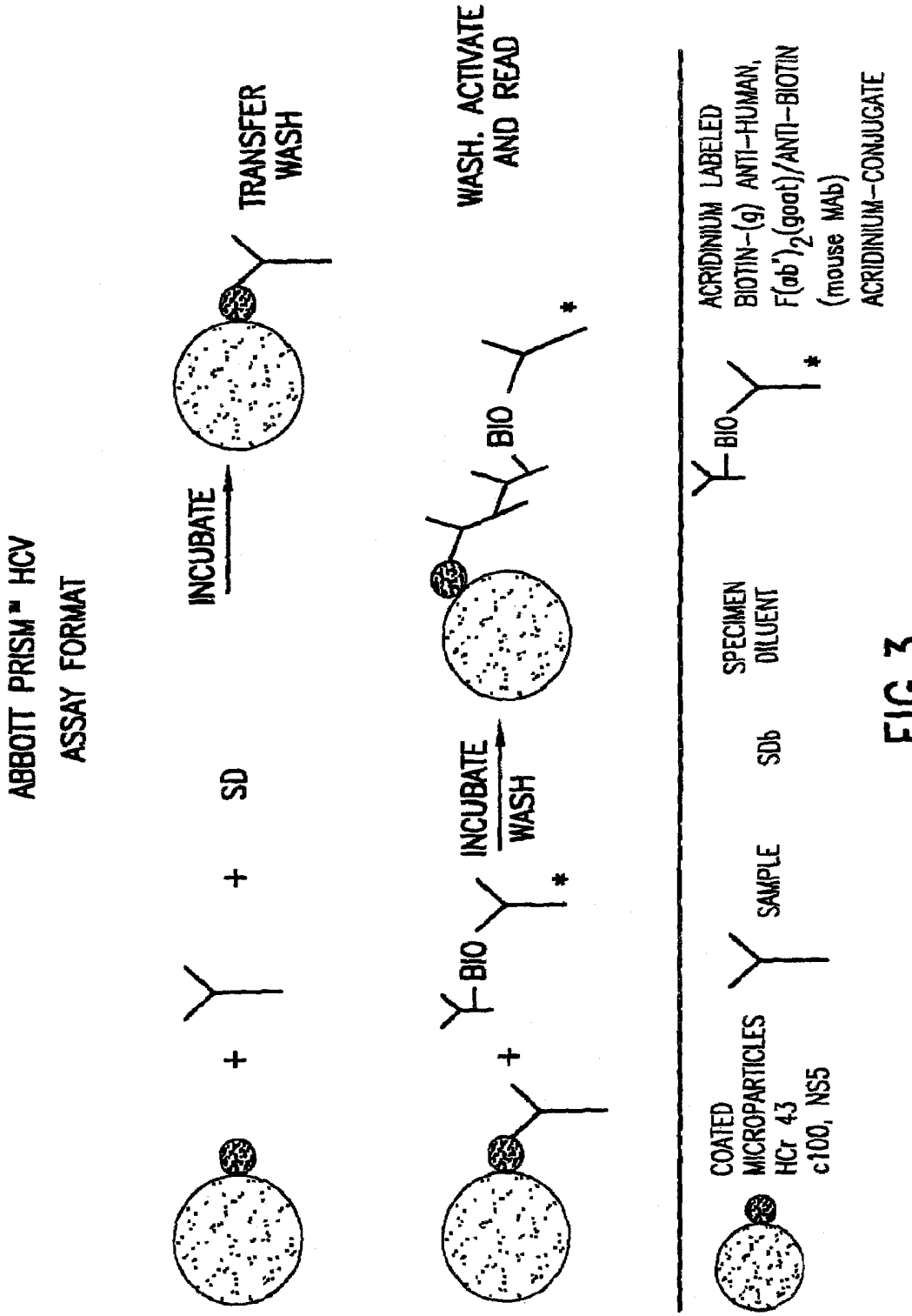
ABBOTT PRISM™ HCV
ASSAY FORMAT
COATED MICROPARTICLES
HCr 43
c100, NS5
SAMPLE
SD
SDb
SPECIMEN DILUENT
INCUBATE
TRANSFER WASH
BIO
INCUBATE
WASH
WASH. ACTIVATE AND READ
ACRIDINIUM LABELED
BIOTIN-(g) ANTI-HUMAN,
F(ab')2(goat)/ANTI-BIOTIN
(mouse MAb)
ACRIDINIUM-CONJUGATE

FIG.3

METHODS FOR THE SIMULTANEOUS DETECTION OF HCV ANTIGENS AND HCV ANTIBODIES

BACKGROUND OF THE INVENTION

1. Technical Field

The subject invention relates to methods for the simultaneous detection of Hepatitis C Virus (HCV) antigens as well as antibodies produced in response to HCV antigens. Furthermore, the subject invention allows one to detect antigens in the early, acute stage of infection, even prior to the development of antibodies, thereby allowing for early detection of infected blood and blood products, and thus improving the safety of the blood supply.

2. Background Information

Recent epidemiological studies indicate that HCV infects more than 170 million people worldwide and that, in more than 50% of the cases, the infection is chronic. In the United States, there are approximately 4 million people infected, and 30,000 new infections are estimated to occur annually (NIH Conference, *Hepatology* Suppl 1:2S (1997)). In addition, HCV is responsible for 8,000–10,000 deaths annually in the United States and is the leading indicator for liver transplantation.

The HCV genome is a single-stranded RNA molecule of positive polarity that is approximately 9400–9500 nucleotides in length. The organization of the coding regions resembles that of other flaviviruses [Major et al., *Hepatology* 25:1527 (1997)] as well as the more recently discovered GB viruses [Muerhoff A S, et al., *J Virol* 69:5621 (1995)]. The HCV genome possesses a large open reading frame (ORF) encoding a polyprotein precursor of 3010 to 3033 amino acids depending on the particular isolate [Choo et al., Proc Natl Acad Sci USA 88:2451 (1991); Grakoui et al., J Virol 67:1385 (1993)}. HCV structural genes (core and envelope) are encoded near the 5'-end of the genome, followed by the proteases and helicase, the helicase cofactor and the replicase. Noncoding regions (NCR), thought to be important in replication, are found at each end of the genome.

HCV infection occurs primarily through parenteral exposure, i.e., through shared needles, by tattooing, or through transfusion of contaminated blood or blood products. Following exposure, the virus enters a susceptible hepatocyte and viral replication occurs. There is an eclipse phase period of approximately 10 days during which time there is no evidence of viral presence, i.e., viral RNA cannot be detected, serum transaminase levels are within normal limits and there is no evidence of an immune response to HCV [Busch et al., *Transfusion* 40:143 (2000)]. Typically, about 10 days following exposure, HCV RNA can be detected, often with viral loads between 100,000–120,000,000 HCV RNA copies per ml of serum. Several weeks later, there is typically an increase in ALT levels indicating inflammation of the liver; antibodies are detected an average of about 70 days after exposure.

One of the preventive measures employed to limit the spread of HCV infections is to screen blood for exposure to HCV, either by the detection of antibodies to HCV or by the detection of viral-specific molecules (e.g. HCV RNA or HCV core proteins) in serum/plasma. Blood or blood products derived from individuals identified as having been exposed to HCV by these tests, are removed from the blood supply and are not utilized for distribution to recipients of blood products. These tests may also be utilized in the clincial setting to diagnose liver disease attributable to HCV infection.

Due to the unavailability of native intact HCV virions, serologic antibody tests have relied on recombinant antigens or synthetic peptides, representing selected fragments of the viral polyprotein. The first generation anti-HCV screening tests were based on detection of antibodies directed against a recombinant protein (HCV genotype la) originating from sequences located in the nonstructural NS-4 protein (C100-3) [Choo et al., *Science* 244:359 (1989); Kuo et al., *Science* 244:362 (1989)]. The first generation assays failed to detect antibodies in approximately 10% of individuals having chronic HCV infection and up to 10–30% of individuals presenting with acute HCV infection. The second generation anti-HCV assays have incorporated recombinant proteins from three different regions of the HCV genome (HCV genotype 1a), including amino acid sequences from the core, NS3, and NS4 protein [Mimms et al., *Lancet* 336:1590 (1990); Bresters et al., *Vox Sang* 62:213 (1992)], allowing a marked improvement over the first generation tests in identifying HCV infected blood donors [Aach et al., *N Engl J Med* 325:1325 (1991); Kleinman et al., *Transfusion* 32:805 (1992)]. The second generation assays detect antibodies in close to 100% of chronic HCV cases [Hino K. *Intervirology* 37:77 (1994)] and in nearly 100% of the acute cases by 12 weeks post infection [Alter et al., *N Engl J Med* 327:1899 (1992); Bresters et al., *Vox Sang* 62:213 (1992)]. The third generation test includes a recombinant protein expressing amino acid sequences from the NS5 region, as well as antigens from the core, NS3 and NS4. Some studies have indicated a slight improvement in sensitivity in comparing the third generation tests to second generation tests [Lee et al., *Transfusion* 35:845 (1995); Courouce et al., 1994. *Transfusion* 34:790–795], but this improvement is largely attributed to changes in the NS3 protein rather than the inclusion of NS5 [Courouce et al., *Lancet* 343:853 (1994)].

In general, the second and third generation HCV antibody tests detect exposure to HCV about 70 days after exposure. Since HCV establishes persistent, and in many cases lifelong, infection, the detection of antibodies to HCV represents a very efficient method for determining exposure to HCV. However, antibody testing alone will frequently fail to detect HCV infected individuals during the first 70 days after their exposure.

The existing HCV antigen tests rely on detecting the presence of the HCV core antigen in serum or plasma. The core (or nucleocapsid) protein comprises the first 191 amino acids of the polyprotein. Two different types of serologic assays have been developed which permit detection of HCV core antigens in serum. One assay format detects HCV core antigens in subjects prior to seroconversion, and is utilized in screening blood donors, while the other assay format detects core antigens only in hepatitis C patients, regardless of their HCV antibody status and is utilized in clinical laboratories to diagnose exposure to HCV or to monitor antiviral therapy.

Recent data on samples obtained during the pre-seroconversion period indicate that the HCV antigen test detects exposure to HCV significantly earlier than antibody testing [Aoyagi et al., *J Clin Microbiol* 37:1802 (1999); Peterson et al., *Vox Sang* 78:80(2000); Dawson et al., *Transfusion, SD*161,40(2000); Muerhoff et al., 7th *International Meeting on Hepatitis C virus and related viruses*, Dec. 3–7, (2000)].], and represents an alternative to nucleic acid testing for detecting exposure to HCV during the pre-seroconversion period. The advantages of HCV antigen detection is that the test is rapid, simple, may not require sample extraction or other pretreatment, and is not as prone to handling errors (e.g., contamination) as may occur in the HCV RNA tests.

In clincial laboratories, the HCV antigen test has comparable sensitivity to the HCV DNA tests in detecting exposure to HCV in patients infected with different HCV genotypes [Dickson et al., *Transplantation* 68:1512 (1999)], and in monitoring antiviral therapy [Tanaka et al., *Hepatology* 32:388 (2000); Tanaka et al., *J Hepatol* 23:742 (1995)]. Thus, HCV core antigen tests present a practical alternative to HCV RNA for screening blood donors or for monitoring antiviral therapy.

The uniqueness of the current invention lies its ability to detection HCV antibodies and HCV antigens simultaneously (see also International Application No. PCT/JP99/04129). This combination test or "combo" assay utilizes antigen detection to identify exposure to HCV during the pre-seroconversion "window period" and antibody detection to identify exposure to HCV after seroconversion.

SUMMARY OF THE INVENTION

The subject invention encompasses a method of simultaneously detecting at least one Hepatitis C Virus (HCV) antigen and at least one HCV antibody in a test sample comprising the steps of: a) contacting the test sample with: 1) at least one HCV viral antigen or portion thereof coated on a solid phase (e.g., a microparticle), for a time and under conditions sufficient for the formation of antibody/antigen complexes and 2) at least one antibody to HCV or portion thereof coated on the solid phase, for a time and under conditions sufficient for the formation of antigen/antibody complexes; b)detecting the antibody/antigen complexes, presence of the complexes indicating presence of at least one HCV antigen in the test sample; and c) detecting the antigen/antibody complexes, presence of the complexes indicating presence of at least one HCV antibody in the test sample. The at least one HCV antigen coated on the solid phase may be, for example, core antigen, NS3, NS4, NS5, and portions (or fragments) thereof. The at least one antibody coated on the solid phase may be, for example, a monoclonal antibody selected from the group consisting of 13-959-270, 14-1269-281, 14-1287-252, 14-153-234, 14-153-462, 14-1705-225, 14-1708-269, 14-1708-403, 14-178-125, 14-188-104, 14-283-112, 14-635-225, 14-726-217, 14-886-216, 14-947-104, 14-945-218, 107-35-54, 110-81-17, 13-975-157, 14-1350-210, C11-3, C11-7, C11-10, C11-14 and C11-15. Further, the at least one monoclonal antibody coated on the solid phase preferably is not reactive with the at least one antigen coated on the solid phase. In particular, the at least one monoclonal antibody may be a HCV anti-core monoclonal antibody and the at least one antigen may be a recombinant HCV core protein. The recombinant core protein does not contain epitopes to which the anti-core monoclonal antibody binds.

Additionally, the present invention includes a method for simultaneously detecting the presence of at least one HCV antigen and at least one HCV antibody in a test sample comprising the steps of: a) contacting the test sample with: 1) at least one HCV viral antigen or portion thereof coated on a solid phase, wherein the solid phase is, for example, a microparticle, for a time and under conditions sufficient for the formation of antibody/antigen complexes and 2) at least one HCV antibody or portion thereof coated on the solid phase, for a time and under conditions sufficient for the formation of antigen/antibody complexes; b) adding a first conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody in (a)(1), wherein the conjugate comprises a second antibody (e.g., mouse anti-human IgG) attached to a label (for example, a chemiluminescent compound) capable of generating a detectable signal and simultaneously adding a second conjugate to the resulting antigen/antibody complexes for a time and under conditions sufficient to allow said second conjugate to bind to the bound antigen in (a)(2), wherein said second conjugate comprises a third antibody (e.g., a monoclonal antibody to anti-HCV core antigen such as C11-10) attached to the label, for example, chemiluminescent compound, capable of generating a detectable signal; and b) detecting the presence of the generated signal, presence of the signal indicating the presence of at least one HCV antigen or at least one HCV antigen in the test sample. Again, the at least one HCV antigen coated on the solid phase may be selected from the group consisting of core antigen, NS3, NS4, NS5, and portions thereof. Further, the at least one antibody coated on the solid phase may be a monoclonal antibody selected from the group consisting of, for example, 13-959-270, 14-1269-281, 14-1287-252, 14-153-234, 14-153-462, 14-1705-225, 14-1708-269, 14-1708-403, 14-178-125, 14-188-104, 14-283-112, 14-635-225, 14-726-217, 14-886-216, 14-947-104, 14-945-218, 13-975-157, 14-1350-210, 107-35-54, 110-81-17, C11-3, C11-7, C11-10, C11-14 and C11-15.

The at least one monoclonal antibody coated on the solid phase is preferably not reactive with the at least one antigen coated on the solid phase.

Also, the present invention encompasses a kit comprising: a) a container containing at least one HCV antigen coated on a solid phase, wherein the solid phase is, for example, a microparticle; and b) a container containing at least one HCV antibody coated on a solid phase, wherein the solid phase is preferably a microparticle.

The present invention also includes a kit comprising: a container containing: 1) at least one HCV antigen coated on a solid phase, wherein the solid phase is preferably a microparticle, and 2) at least one HCV antibody, coated on the solid phase.

The kit may further comprise at least one conjugate comprising a signal-generating compound attached to a HCV antigen or HCV antibody. The signal-generating compound may be, for example, acridinium.

Additionally, the present invention includes a method of detecting HCV antigen in a test sample comprising the steps of: a) contacting the test sample with at least one HCV antibody (e.g., monoclonal) coated on a solid phase, wherein said solid phase is a microparticle, for a time and under conditions sufficient for the formation of antibody/antigen complexes; and b) detecting the presence of antibody/antigen complexes, presence of the complexes indicating presence of antigen in the test sample.

The invention also encompasses a method of detecting HCV antigen in a test sample comprising the steps of: a) contacting the test sample with at least one HCV antibody (e.g., monoclonal) coated on a solid phase, wherein the solid phase is, preferably, a microparticle, for a time and under conditions sufficient for the formation of antibody/antigen complexes; b) adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound at least one antibody, wherein said conjugate comprises a second antibody attached to label, for example, a chemiluminescent compound capable of generating a detectable signal; and c) detecting the signal generated by the label, for example, chemiluminescent compound, a signal generated by the label indicating the presence of antigen in the test sample.

Also, the present invention includes a recombinant protein comprising an amino acid sequence selected from the group consisting of, for example, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:12 and SEQ ID NO:16 as well as comprises conservative amino acid substitutions of these sequences. (A conservative substitution is defined as one or more amino acid substitutions in a sequence which do not change the function of the sequence.) The present invention also includes a recombinant protein comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of, for example, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11 and SEQ ID NO:15. (Substitutions, deletions and additions within the sequences which do not affect functionally affect the protein encoded by the sequence are also considered to be within the scope of the present invention.) Additionally, the present invention includes a vector or construct comprising a nucleotide sequence selected from the group consisting of, for example, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11 and SEQ ID NO:15. The invention also includes a host cell comprising the vector or construct.

Furthermore, the present invention includes an immunoassay which may simultaneously detect at least one HCV antigen or at least one HCV in a test sample.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates the Abbott PRISM® HCV Ab assay format. The assay uses a 2-step format, which consists of microparticles coated with recombinant HCV antigens from the core, NS3, NS4 and NS5 regions of the HCV genome. These microparticles, when combined with the donor specimen, a diluent, and a complex of goat polyclonal anti-human $F(ab')_2$ fragment/murine monoclonal anti-biotin:Acridinium conjugate yield an amount of photons representing a qualitative measurement of anti-HCV antibodies in the specimen when triggered with the PRISM Activator solution.

FIG. **2** illustrates the HCV Ag assay format. This assay also uses a 2-step format. When microparticles coated with HCV Mab (e.g. c11-14) are combined with the donor specimen, a diluent and ac ridinium-labeled Mab (e.g. acridinium labeled c11-10), an amount of photons representing a qualitative measurement of anti-HCV antigens in the specimen will result. The measured amount of photons indicates the amount of HCV antigens in the specimen when triggered with the PRISM Activator solution.

FIG. **3** illustates the HCV Ag/Ab combo assay format. The assay uses a 2-step format. When HCV recombinant antigen and monoclonal antibody blended microparticles (e.g. HCV peptide from the core, and recombinant antigens from the NS3, NS4 and NS5 regions of the HCV genome blended with microparticles coated with c11-14) are combined with the donor specimen, a diluent and blended Acridinium-labeled Mabs (e.g. acridinium labeled c11-10 and acridinylated mouse-anti-human IgG), an amount of photons representing a qualitative measurement of anti-HCV antigens, or anti-HCV antibodies or both in the specimen will result when triggered with PRISM Activator solution.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to various methods which may be utilized in order to simultaneously detect antigens of HCV and antibodies to HCV in a biological sample. Thus, if an individual has either developed specific antibodies to HCV and/or has HCV specific antigens in the biological sample tested, the methods of the present invention will yield a positive result. Such results may be used, for example, to diagnose the patient in terms of presence and status of infection (i.e., acute or chronic) as well as to determine the suitability of a donor blood or blood product sample for transfusion.

Also, the present invention overcomes the problems associated with the "window period" (i.e., 50–60 days post infection) wherein an individual may be infected with HCV but may not have developed antibodies yet. Such individuals may transmit HCV to others during this period. Thus, by detecting HCV during this "window period", the present invention allows for a quick diagnosis of HCV, as opposed to waiting for the development of antibodies, and prevents contamination of the blood supply.

In one embodiment of the present invention, HCV viral antigens (e.g., core, N3, N4 and N5), or portions thereof, are coated on a solid phase (or are in a liquid phase). The test or biological sample (e.g., serum, plasma, urine, etc.) is then contacted with the solid phase. If antibodies are present in the sample, such antibodies bind to the antigens on the solid phase and are then detected by either a direct or indirect method. The direct method comprises simply detecting presence of the complex itself and thus presence of the antibodies. In the indirect method, a conjugate is added to the bound antibody. The conjugate comprises a second antibody, which binds to the first bound antibody, attached to a signal-generating compound or label. Should the second antibody bind to a bound first antibody, the signal-generating compound generates a measurable signal. Such signal then indicates presence of the first antibody in the test sample.

Examples of solid phases used in diagnostic immunoassays are porous and non-porous materials, latex particles, magnetic particles, microparticles (see U.S. Pat. No. 5,705,330), beads, membranes, microtiter wells and plastic tubes. The choice of solid phase material and method of labeling the antigen or antibody present in the conjugate, if desired, is determined based upon desired assay format performance characteristics.

As noted above, the conjugate (or indicator reagent) will comprise an antibody (or perhaps anti-antibody, depending upon the assay), attached to a signal-generating compound or label. This signal-generating compound or "label" is in itself detectable or may be reacted with one or more additional compounds to generate a detectable product. Examples of signal-generating compounds include chromogens, radioisotopes (e.g., 125I, 131I, 32P, 3H, 35S and 14C), chemiluminescent compounds (e.g., acridinium), particles (visible or fluorescent), nucleic acids, complexing agents, or catalysts such as enzymes (e.g., alkaline phosphatase, acid phosphatase, horseradish peroxidase, beta-galactosidase and ribonuclease). In the case of enzyme use (e.g., alkaline phosphatase or horseradish peroxidase), addition of a chromo-, fluro-, or lumo-genic substrate results in generation of a detectable signal. Other detection systems such as time-resolved fluorescence, internal-reflection fluorescence, amplification (e.g., polymerase chain reaction) and Raman spectroscopy are also useful.

Examples of biological fluids which may be tested by the above immunoassays include plama, serum, cerebrospinal fluid, saliva, tears, nasal washes or aqueous extracts of tissues and cells.

At the same time as the antibodies are being detected, HCV antigens are also being detected; thus, the present invention obviates the need for the running of two different tests. This is accomplished by exposing the test sample to a

solid phase (or liquid phase) coated with specific antibodies to HCV (e.g., human or animal monoclonal antibodies to core, polyclonal antibodies, chimeric antibodies, etc.). Antigens, if present in the sample, bind to the solid phase and may then be detected by a direct or indirect method as described above. More specifically, the indirect method involves the addition of a conjugate comprising a second antibody (which binds to the bound antigen) attached to a label or signal-generating compound. When the second antibody binds to the bound antigen, a detectable signal is then generated indicating presence of HCV antigen in the test sample.

The antibodies which are coated on the solid phase as well as the "second antibody" may be, as noted above, monoclonal antibodies or polyclonal antibodies. For example, if one chooses to utilize monoclonal antibodies, they may be selected from 13-959-270, 14-1269-281, 14-1287-252, 14-153-234, 14-153-462, 14-1705-225, 14-1708-269, 14-1708-403, 14-178-125, 14-188-104, 14-283-112, 14-635-225, 14-726-217, 14-886-216, 14-947-104 and 14-**945-218**, The following anti-core monoclonal antibodies may also be utilized for purposes of the present invention: 107-35-54, 110-81-17, 13-975-157, 14-1350-210 (see U.S. Pat. No. 5,753,430) and Tonen HCV core monoclonals C11-3, 7, 10, 14 and 15 (see PCT Application WO 099/06836), all of which are available from the American Type Culture Collection. 10801 University Boulevard, Manassas, Va. **20110-2209**. (For a discussion of the manner in which monoclonal antibodies may be created, see Kohler and Milstein, Nature (1975) 256:494, and reviewed in Monoclonal Hybridoma Antibodies: Techniques and Applications, ed. Hurrell (CRC Press, Inc., 1982); see also J. W. Goding in *Monoclonal Antibodies: Principles and Practice* (Academic Press, N.Y., 1983; see also U.S. Pat. No. 5,753,430).

It should be noted that HCV core protein may be one possible target of the HCV antigen portion of the assay. More specifically, the detection of the core protein is accomplished by using monoclonal antibodies directed towards epitopes within the core protein. These anti-core monoclonals are placed on the solid phase and facilitate the capture of core antigen proteins from the test sample. For detection of HCV antibodies in the test sample, recombinant HCV core protein is also placed on the solid phase. It should be noted however that there are significant problems associated with the use of a single protein as the target for an antigen test and as the capture reagent for antibody detection, namely there is significant "cross-reactivity" between the core antigen and the anti-core monoclonal antibodies coated onto the solid phase(s). This results in a false positive signal, even in the absence of the test sample, since the monoclonal antibodies will bind to epitopes present on the recombinant protein.

In order to avoid such cross-reactivity, the core protein used in the antibody detection portion of the assay may be modified such that the ability of the anti-core monoclonals to bind HCV core is eliminated. Such modification may be achieved by use of recombinant DNA technology in which the epitope region (i.e., the short sequence of amino acids needed for monoclonal antibody binding) is eliminated or modified. Thus, use of the modified recombinant core protein would consequently maintain several human epitopes to which antibodies present in the serum of infected individuals would bind; however, the anti-core monoclonal antibodies used for antigen capture would not bind the modified protein. Alternatively, one could replace the HCV core recombinant protein with polypeptides that include sequences known to bind to antibodies present in the serum of most infected individuals, but do not include sequences containing the epitopes recognized by the anti-core monoclonals used to detect HCV core antigens.

More specifically, as noted above, in order to avoid cross-reactivity, one may use core antigens for antibody detection in the assay. In particular, in the present invention, the solid phase may be coated with nonstructural proteins (NS) 3, 4 and/or 5 (i.e., NS3, NS4 and/or NS5) and/or the core protein. Alternatively, in the present invention, the solid phase may be coated with any of the above-mentioned HCV proteins, or segments or portions thereof, either individually or in combination (for antibody detection). The antigens used for coating the solid phase may be generated as a contiguous recombinant protein, expressed as recombinant proteins, either as a single entity or as discrete entities, or as synthetic peptides designed either as a single entity or discrete entities.

With respect to detection of antigens in the present invention, as noted above, the monoclonal or polyclonal antibodies coated on the solid phase must not recognize the core antigens used on the solid phase (for antibody detection). Thus, for example, in the present invention, one may use the full antibody or a fragment thereof. (For purposes of the present invention, a "fragment" or "portion" of an antibody is defined as a subunit of the antibody which reacts in the same manner, functionally, as the full antibody with respect to binding properties.)

Additionally, it should also be noted that the initial capture antibody (for detecting HCV antigens) used in the immunoassay may be covalently or non-covalently (e.g., ionic, hydrophobic, etc.) attached to the solid phase. Linking agents for covalent attachment are known in the art and may be part of the solid phase or derivatized to it prior to coating.

The second manner in which to use the solid phase for detecting HCV antibodies involves elimination of the core antigens entirely. For example, the solid phase is coated with NS3, NS4 and/or NS5 and a substitute for the core protein or regions thereof (e.g., E2). In contrast, the antibodies coated on the solid phase for detection of antigen are directed against the core protein of HCV.

Other assay formats which may be used for purposes of the present invention, in order to simultaneously detect antigens and antibodies include, for example, Dual assay strip blots, a rapid test, a Western blot, as well as the use of paramagnetic particles in, for example, an Architect® assay (Frank Quinn, The Immunoassay Handbook, Second edition, edited by David Wild, pages 363–367, 2001). Such formats are known to those of ordinary skill in the art.

It should also be noted that the assays of the present invention may also be used to solely detect HCV antigens or HCV antibodies, rather than both, if desired. Certainly, if one desires to establish that an infection initially exists, one may simply want to determine the presence of antigen in a test sample such as during the "window period". On the other hand, if one wants to establish the stage of infection (e.g., acute versus chronic), one may wish to look for the presence of antibodies and titer thereof.

It should also be noted that the elements of the assay described above are particularly suitable for use in the form of a kit. The kit may also comprise one container such as vial, bottles or strip, with each container with a pre-set solid phase, and other containers containing the respective conjugates. These kits may also contain vials or containers of other reagents needed for performing the assay, such as washing, processing and indicator reagents.

The present invention may be illustrated by the use of the following non-limiting examples:

EXAMPLE I

Mapping of HCV Core Epitopes Recognized by Monoclonal Antibodies

To determine the region within the HCV core protein to which each of the monoclonal antibodies binds, a series of overlapping, biotinylated peptides were synthesized (Table I). These peptides were used to develop EIAs, as described below. It should be noted that all monoclonals were able to detect a recombinant HCV core fusion protein using EIA methodologies similar to that described below (data not shown).

TABLE I

HCV-Core Derived Peptides

Peptide	Sequence	Core Region Represented
A	MSTNPKPQKKNKRNTNRR	1–18
B	NKRNTNRRPQDVKFPGGG	11–28
C	DVKFPGGGQIVGGVYLLP	21–38
D	VGGVYLLPRRGPRLGVRA	31–48
E	GPRLGVRATRKTSERSQP	41–58
F	KTSERSQPRGRRQPIPKA	51–68
G	RRQPIPKARRPEGRTWAQ	61–78
H	PEGRTWAQPGYPWPLYGN	71–88
I	QYPWPLYGNEGCGWAGWLL	81–98
J	CGWAGWLLSPRGSRPSW	91–107
1	WLLSPRGSRPSWGPTDPRRRSRNLG	96–120
2	SWGPTDPRRRSRNLGKVIDTLTCGF	106–130
3	SRNLGKVIDTLTCGFADLMGYIPLV	116–140
4	LTCGFADLMGYIPLVGAPLGGAARA	126–150
5	YIPLVGAPLGGAARALAHGVRVLED	136–160
6	GAARALAHGVRVLEDGVNYATGNLP	146–170
7	LEDGVNYATGNLPGCSFSIFLLA	158–180
8	LPGCSFSIFLLALLSCLTVPASA	169–191

Coating of polystyrene beads: One quarter-inch polystyrene beads were used as the solid phase for the peptide EIAs. Prior to coating, beads were washed with 15% isopropanol (in water) at room temperature for 30 minutes without agitation. Isopropanol was removed and the beads were rinsed once with deionized water. The washed beads were then added to a vial containing the peptide diluted to 5 μg/ml in 0.1 M sodium phosphate, pH 7.5 buffer (0.233 ml per bead). Beads were incubated at 56° C. for 2 hours with end-over-end mixing. Beads were then washed three times with PBS and then incubated in PBS containing 0.1% Triton X-100 at 40° C. for 1 hour with end-over-end mixing. They were again washed three times in PBS and then incubated at 40° C. in 5% BSA/PBS for 1 hour end-over-end mixing. Beads were washed four times with PBS and then incubated at room temperature in PBS containing 5% sucrose without mixing for 20 minutes. Sucrose buffer was removed and beads air-dried. Coated beads were stored desiccated at 4° C.

Bead coating validation: To determine whether the biotinylated peptides were actually coated onto the beads, an assay was performed in which beads were incubated in buffer containing horseradish peroxidase-labeled streptavidin (200–400 ng/ml). The beads were then washed with deionized water and substrate added. Product was detected by absorbance at 492 nm. All peptides in Table I were shown to be coated onto the polystyrene beads by this assay (data not shown).

HCV peptide EIAs: Monoclonal antibodies generated against a recombinant HCV core protein (see Example W) were tested for their ability to bind to each of peptide-coated beads as follows: monoclonal antibodies were diluted to 50 ng/ml in sample diluent buffer (Tris buffer containing 20% goat serum, 10% calf serum, 0.2% Triton X-100 and sodium azide) of which 0.2 ml was added into a reaction well containing the peptide-coated bead and incubated at room temperature for 2 hours with mixing. Beads were then washed with deionized water followed by the addition of 0.2 ml of peroxidase-labeled goat anti-mouse IgG (0.3 μg/ml). Beads were incubated at room temperature for 60 minutes with mixing. Beads were washed with deionized water and then transferred into plastic tubes to which 0.3 ml of OPD (0.3% O-phenylenediamine-2-HCl in citrate buffer containing 0.02% H_2O_2) substrate was added and incubated in the dark at room temperature for 30 min without mixing. Reaction was quenched by the addition of 1 ml of 1N H2SO4 and the OD at 492 nm determined. The absorbance is directly proportional to the amount of antibody bound to the bead.

Peptide mapping of monoclonals: Using the assay as described above, each of the monoclonals were assayed for their ability to bind each of the HCV-core-derived peptide coated beads. When a monoclonal antibody was found to bind to a specific peptide-coated bead, 10-fold serial dilutions of the monoclonal antibody were made which were then assayed for binding to the same peptide. This allowed the determination of binding specificity for each monoclonal antibody. Results shown in Table II indicate the lowest dilution of monoclonal antibody that exhibited binding (absorbance at least 3-times background).

TABLE II

Anti-core monoclonal peptide mapping

	Peptide								
Monoclonal	A aa1-18	B aa11-28	C aa21-37	D aa31-48	E aa41-57	F aa51-68	G aa61-78	H aa71-88	I aa81-98
14-1350-210 A07	—	—	—	—	—	—	—	—	—

TABLE II-continued

		Anti-core monoclonal peptide mapping								
13-975-157	A08	—	—	—	—	—	—	—	—	—
13-959-270	A09	—	—	—	—	—	—	—	—	—
110-81-17	A15	—	—	—	—	—	—	—	—	—
107-35-54	A04	—	—	—	—	—	—	—	—	—
14-1708-269	A269	—	—	—	—	—	5 ng/ml	—	—	—
14-1705-255	A10	—	—	—	—	—	500 pq/ml	—	—	—
14-1287-252	A12	—	—	—	—	—	5 ng/ml	—	—	—
14-1269-281	A03	—	—	—	—	—	50 ng/ml	—	—	—
14-947-104	A16	—	—	—	—	—	500 pq/ml	—	—	—
14-945-218	A218	—	—	—	—	—	—	—	—	—
14-886-216	A14	—	—	—	—	—	—	—	—	—
14-726-217	A06	—	—	—	—	—	50 ng/ml	—	—	—
14-635-225	A05	—	—	—	—	—	—	—	—	—
14-283-112	A112	—	—	—	—	—	5 ng/ml	—	—	—
14-188-104	A11	—	—	—	—	—	5 ng/ml	—	—	—
14-178-125	A13	—	—	—	—	—	500 pg/ml	—	—	—
14-153-234	A234	—	—	—	—	—	500 pg/ml	—	—	—
C11-3	C11-3	—	—	—	—	—	—	—	—	—
C11-7	C11-7	—	—	—	—	—	—	—	—	—
C11-10	C11-10	—	—	—	—	—	—	—	—	—
C11-14	C11-14	—	—	—	—	—	—	—	—	—
C11-15	C11-15	—	50 ng/ml	—	—	—	—	—	—	—

		Peptide							
Monoclonal		1 aa96-120	2 aa106-130	3 aa116-140	4 aa126-150	5 aa136-160	6 aa146-170	7 aa158-180	8 aa169-191
14-1350-210	A07	—	—	—	—	—	—	—	—
13-975-157	A08	—	50 ng/ml	—	—	—	—	—	—
13-959-270	A09	—	—	—	—	—	—	—	—
110-81-17	A15	50 ng/ml	5 ng/ml	—	—	—	—	—	—
107-35-54	A04	50 ng/ml	—	—	—	—	—	—	—
14-1708-269	A269	—	—	—	—	—	—	—	—
14-1705-255	A10	—	—	—	—	—	—	—	—
14-1287-252	A12	—	—	—	—	—	—	—	—
14-1269-281	A03	—	—	—	—	—	—	—	—
14-947-104	A16	—	—	—	—	—	—	—	—
14-945-218	A218	—	—	—	—	—	—	—	—
14-886-216	A14	—	—	—	—	—	—	—	—
14-726-217	A06	—	—	—	—	—	—	—	—
14-635-225	A05	—	—	—	—	—	—	—	—
14-283-112	A112	—	—	—	—	—	—	—	—
14-188-104	A11	—	—	—	—	—	—	—	—
14-178-125	A13	—	—	—	—	—	—	—	—
14-153-234	A234	—	—	—	—	—	—	—	—
C11-3	C11-3	5 ng/ml	—	—	—	—	—	—	—
C11-7	C11-7	—	50 pg/ml	5 ng/ml	—	—	—	—	—
C11-10	C11-10	—	—	—	—	—	—	—	—
C11-14	C11-14	—	—	—	—	—	—	—	—
C11-15	C11-15	—	—	—	—	—	—	—	—

EXAMPLE II

Epitope Mapping of Monoclonal Antibodies

A. Preparation of HCV Gene Fragment Library.

A plasmid containing nucleotides 14–5294 of the H strain of HCV (N. Ogata, H. Alter, R. Miller, and R. Purcell, *Proc. Natl. Acad. Sci. USA* 88:3392–3396 (1991)) in pGEM-9Zf (−) (Promega Corp., Madison, Wis.) was partially digested using DNase I by the following method in order to obtain random epitope-encoding fragments. Five μg aliquots of plasmid DNA were incubated at 15° C. for 10 minutes in 0.5 M Tris-HCl pH 7.6, 10 mM $MnCl_2$ in the presence of anywhere from 0.1 to 0.7 units of DNase I. Aliquots from each digestion were analyzed by agarose gel electrophoresis. The two digestion mixtures containing 0.6 and 0.7 units of DNase I were found to give the largest amount of fragments in the 50–200 bp range. These two mixtures were pooled and extracted one time with an equal volume of phenol-chloroform (1:1, v/v) then precipitated by the addition of one tenth volume 3 M sodium acetate and 2.5 volumes 100% ethanol followed by centrifugation at 14,000×g for 10 minutes. The ends of the DNA molecules were then made blunt using the PCR Polishing kit (Stratagene, Inc., La Jolla, Calif.) as per manufacturer's directions. The DNA was again extracted and precipitated as described above, followed by ligation to a double-stranded adaptor in a 10 μl reaction volume using a T4 DNA ligase kit (Stratagene, Inc., La Jolla, Calif.) as directed by the manufacturer. The sequence of this double stranded adaptor was:

```
5'-GATCGCTCGAATTCCTCG-3'     (SEQUENCE ID NO:1)

3'-TTCTAGCGAGCTTAAGGAGC-5'   (SEQUENCE ID NO:2)
```

The sense-strand oligonucleotide of the adapator (SEQ ID NO:1) was then used as a primer in a PCR reaction such that all DNAs were amplified independent of their sequence.

This method is a modification of that described by A. Akowitz and L. Manuelidis, *Gene* 81:295–306 (1989) and G. Reyes and J. Kim, *Mol. Cell. Probes* 5:473–481 (1991). PCR was performed in the presence of the sense-strand oligonucleotide primer at a final concentration of 1 μM in a reaction volume of 100 μl using the GeneAmp Gold PCR kit (PE Applied Biosystems, Foster City, Calif.) as directed by the manufacturer in a PE-9600 thermocycler. A pre-incubation at 94° C. for 8 min was followed by twenty-five cycles of PCR as follows: denaturation at 94° C. for 20 seconds, annealing at 55° C. for 30 seconds, and extension at 72° C. for 1.0 min. This was followed by a final extension step at 72° C. for 10 min. The PCR product was extracted and precipitated as described above. The entire PCR was run on a 1.2%. agarose gel and a gel slice containing DNA fragments between approximately 70 and 250 bp was removed. The DNA was extracted from the gel slice using the QIAEX II kit (QIAGEN, Inc., Valencia, Calif.) as per manufacturer's directions. The DNA was digested with the restriction enzyme EcoRI (New England Biolabs, Beverly, Mass.) as directed by the manufacturer. The digested DNA was then extracted and precipitated as described above.

T7Select10-3b (Novagen, Inc., Madison, Wis.) was digested with EcoRI and dephosphorylated with calf intestinal alkaline phosphatase (New England Biolabs, Beverly, Mass.) as directed by the manufacturer. Size-selected digested DNA fragments (30 ng) (supra) were ligated with 0.5 μg digested T7Select10-3b in a 5 μl reaction volume at 16° C. overnight. The entire ligate was packaged into phage heads using T7Select packaging extract (Novagen, Inc., Madison, Wis.) and titered as directed by the manufacturer. The resulting unamplified library contained a total of 3.9×10^6 members (PFU). The packaged phage were amplified by liquid lysate amplification in *E. coli* BLT5403 (20 ml culture) as directed by the *T7 Select System Manual* (Novagen, Inc., Madison, Wis.). The amplified library had a titer of 1.3×10^{11} PFU per ml.

B. Biopanning of HCV Gene Fragment Library.

Each monoclonal antibody (20 μg) that was to be used for biopanning was incubated at 4° C. for 4 hours on an end-over-end rocker in 300 μl blocking buffer (2% BSA, 3% nonfat dry milk, 0.2% Tween 20, 0.02% sodium azide in phosphate-buffered saline). During the incubation of the monoclonal antibody, an aliquot of the amplified HCV gene fragment library (supra) containing approximately 10^{11} phage was precipitated as follows: 1/10 volume of 5 M NaCl was added to the phage, mixed thoroughly, followed by addition of 1/6 volume polyethylene glycol (MW 8000), mixed thoroughly again, and incubated on ice for 1–2 hours. The phage were centrifuged at 6000×g for 10 min at room temp, all supernatant was removed and the phage pellet was vigorously resuspended in 120 μl buffer containing 1 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA. The phage were added to the pre-incubated monoclonal antibody and incubated at 4° C. overnight on an end-over-end rocker.

The next morning, the antibody-phage complexes were captured on paramagnetic particles coupled to goat anti-mouse IgG (Fc specific) as follows. A 0.2 ml aliquot of Goat Anti-Mouse IgG Fc BioMag particles (Polysciences, Inc., Warrington, Pa.) was washed three times with 0.4 ml 0.1% Tween 20, 0.1% BSA, 0.02% sodium azide in phosphate-buffered saline (PBS) by gentle vortexing followed by capture on a magnetic stand for 0.5–1 minute. The supernatant was removed carefully without disturbing the particles. Particles were then resuspended in the IgG-phage from overnight incubation above and incubated at room temperature on an end-over-end rocker for 3 hours. Particles were washed six times as above using 6.0 ml 0.5% Tween 20, 0.1% BSA in PBS per wash. Bound phage were eluted using 0.2 ml 0.1% Tween 20, 0.1% BSA, 1.0% SDS in PBS at room temperature on an end-over-end rocker for 90 minutes. The tube was placed on a magnetic stand for 1–2 minutes, after which the supernatant containing the eluted phage was removed to a clean tube. The sample containing eluted phage was titered as directed in the *T7Select System Manual.*

The eluted phage was amplified as follows. Ten ml LB Broth (Gibco BRL, Gaithersburg, Md.) plus 100 μg/ml ampicillin was inoculated with *E. coli* BLT5403 and incubated at 37° C. overnight with shaking. The following morning, 35 ml LB Broth plus 100 μg/ml ampicillin, 1× M9 salts, 0.4% glucose, 1 mM $MgSO_4$ was inoculated with 0.2 ml of the overnight culture and incubated at 370C with shaking until the A600 absorbance was 0.5–0.6. Eluted phage (185 μl) from first round biopanning (supra) was added and incubation at 37° C. was continued for 1.5–2 hours, until the A600 absorbance of the culture had dropped to approximately 0.5, indicating lysis. The culture was centrifuged at 8000×g for 10 minutes and the supernatant was removed to a clean tube and stored at 4° C. The culture supernatant was titered as directed in the *T7 Select System Manual.*

One to two subsequent rounds of biopanning and amplification were performed as above with the following modifications. After pre-blocking the monoclonal antibody for 4 hours at 4° C., 150 μl amplified phage from the previous round of biopanning was added instead of 10^{11} PEG-precipitated phage from the starting library. In addition, after biopanning, a 20 ml culture rather than a 35 ml culture was used to amplify the eluted phage, and 100 μl rather than 185 μl of eluted phage was added to the culture.

C. Selection and Sequencing of HCV Core-Containing Clones.

A DNA fragment containing a region of the HCV genome that encodes amino acids 1–173 of the HCV nucleocapsid protein was utilized as a hybridization probe. This region was chosen because all of the monoclonal antibodies analyzed in the biopanning experiments recognize epitopes in the HCV core protein. Phage resulting from 2–3 rounds of biopanning and amplification were plated on *E. coli* BLT5403 and incubated at 37° C. until plaques formed. DNA was transferred onto Hybond-N+ membranes (Amersham Life Sciences, Inc., Arlington Heights, Ill.) denatured, neutralized, and UV cross-linked as described by the manufacturer. The membranes were pre-hybridized, hybridized with the HCV nucleocapsid gene ^{32}P-labeled probe, washed and exposed as described and known in the art. Individual hybridizing plaques were isolated and the inserts were amplified by PCR using T7SelectUP and T7SelectDOWN primers (Novagen, Inc., Madison, Wis.) as directed in the *T7Select System Manual.* For each monoclonal antibody, 30–50 independent hybridizing plaques were amplified and then purified using the QIAquick PCR purification kit (Qiagen, Inc., Chatsworth, Calif.). Purified PCR products were sequenced directly on an ABI Model 377 DNA Sequencer using the ABI Big Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) and the T7SelectUP primer. All of the sequences resulting from biopanning with a particular monoclonal antibody were aligned with the HCV nucleocapsid gene sequence and the minimum region of overlap among all clones was identified. This overlap region defined the epitope recognized by the

monoclonal antibody. The epitopes recognized by several monoclonal antibodies that were identified using this method are shown in TABLE III.

TABLE III

Monoclonal Antibody	Region of HCV Core Recognized
C11-15	Amino acids 19–27
C11-10	Amino acids 32–36
C11-14	Amino acids 45–50
C11-3	Amino acids 104–110
C11-7	Amino acids 112–124
14-635-225	Amino acids 49–53
14-153-462	Amino acids 50–63
14-726-217	Amino acids 57–63
14-178-125	Amino acids 59–64
14-1269-281	Amino acids 59–64
14-947-104	Amino acids 59–64
14-188-104	Amino acids 59–64
14-1708-269	Amino acids 59–64
107-35-54	Amino acids 102–109

EXAMPLE III

Construction of Recombinant Antigens for Use in an HCV Core Antibody/Antigen Combination Assay

A. Background.

The human immune response to Hepatitis C Virus (HCV) core is for the most part exclusive to the N-terminal half of the native protein. Multiple epitopes (regions comprising a defined number of amino acids, usually <10) have been identified within the first 115 amino acids of the native protein (Sallberg et al). Therefore, recombinant antigens utilized in assays for the detection of human anti-core antibodies present in the serum of infected individuals need only contain this portion of the native protein. Conversely, in vitro assays for the detection of HCV core protein utilize murine monoclonal antibodies to capture and detect native core protein also present in the serum of infected individuals. Combination assays for the simultaneous detection of both core antigen and human anti-core antibody in a single assay combine the two assay formats. In this case, a recombinant core antigen is necessary that will be recognized by human anti-core antibodies present in the serum, while escaping recognition by the murine monoclonal antibodies used to capture and detect native core antigen also present in serum. Such recombinants can be constructed by eliminating small regions (1–30 or more amino acids) within the core antigen, thus disrupting or eliminating the epitope(s) recognized by the murine monoclonal antibodies while at the same time leaving undisturbed numerous other epitopes that will allow human anti-core antibody detection.

B. Antigen Construction.

The first of these antigens constructed contained HCV amino acids 8–100 in which amino acids 32–50 were deleted from the recombinant. In this manner, antibodies C11-10 and C11-14, which bind to epitopes at amino acids 32–36 and 45–50, respectively (Example TP-M), will not bind to the resulting recombinant antigen. A plasmid containing a bacterial codon-optimized version of nucleotides 342–791 from the H strain of HCV (Ogata, 1991) was used as template for the PCR. Oligonucleotide primers designed to this sequence were utilized to amplify two distinct fragments (1=SEQ ID 1 and 2; 2=SEQ ID 3 and 4) of the HCV core antigen, one upstream of the deletion and the other downstream of the deletion. Additionally, the oligonucleotide primers flanking the deletion on each fragment (SEQ ID 2 and 3) contained regions of overlap with one another. PCR was performed in a PE-9600 thermocycler in the presence of 0.5 μM of each oligonucleotide primer and 3–5 pg of plasmid template using the TaKaRa LA Taq PCR Kit (Pan Vera, Corp., Madison, Wis.) as per manufacturer's instructions (50 μl volume). A pre-incubation at 94° C. for 1 minute was followed by 35 cycles of PCR (denaturation at 94° C. for 20 seconds; annealing at 50° C. for 30 seconds; extension at 72° C. for 30 seconds), which was then followed by a final extension at 72° C. for 10 minutes. Following the independent amplification of the two fragments, each was purified using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, Calif.) and eluted into 25 μl of water. A second round of PCR was then performed as supra for 10 cycles to tether the two purified fragments (1 μl each in a 20 μl volume reaction) to one another at the regions of overlap within the oligonucleotide primers (SEQ ID 2 and 3). Finally, the product of the second PCR was amplified in a third PCR for 35 cycles utilizing 0.5 μM of the flanking oligonucleotide primers (SEQ ID 1 and 4) and the conditions described supra (100 μl volume).

C. Recombinant Expression.

The product of the third PCR was purified using the QIAquick PCR Purification Kit, then digested with the restriction enzymes EcoRI and BamHI, digestion sites that were incorporated into the flanking oligonucleotide primers (SEQ ID 1 and 4). The fragment encoding the recombinant was ligated into the bacterial expression vector pJO200 that had been similarly digested with EcoRI and BamHI using the pGEM-T Easy Ligation Kit (Promega, Madison, Wis.), then transformed into XL1-Blue competent cells (Stratagene, La Jolla, Calif.). After selection of clones containing the appropriately sized inserts, the nucleotide sequence of the recombinant was confirmed (SEQ ID 5), exhibiting the deduced amino acid sequence (SEQ ID 6). (See U.S. Pat. Nos. 5,322,769 and 6,172,189 for a description of the expression of recombinant proteins.)

D. Other Recombinants.

Several other HCV core recombinants have been constructed, cloned and expressed in a manner identical to that described supra. The first of these (see nucleotide sequence SEQ ID NO. 7 and corresponding amino acid sequence SEQ ID NO. 8) contains HCV amino acids 8–100 in which amino acids 33–35 and 46–49 have been deleted. The oligonucleotide primers utilized to amplify the two fragments in the first PCR were SEQ ID 1 and 9, and SEQ ID 10 and 4, respectively, followed by final amplification in the third PCR with SEQ ID 1 and 4. The second of these recombinants (see nucleotide sequence SEQ ID NO. 11 and corresponding amino acid sequence SEQ ID NO. 12) encodes HCV amino acids 8–100 in which the leucine reside at amino acid 36 has been substituted with valine, and the arginine residue at amino acid 47 has been substituted with leucine. The oligonucleotide primers utilized to amplify the two fragments in the first PCR were SEQ ID 1 and 13, and SEQ ID 14 and 4, respectively, followed by final amplification in the third PCR with SEQ ID 1 and 4. Finally, a recombinant (see nucleotide sequence SEQ ID NO: 15 and corresponding amino acid SEQ ID NO:16) encoding HCV amino acids 8–100 was constructed using oligonucleotide primers SEQ ID 1 and 4 in a single PCR reaction.

SEQ ID NO:1

5'HO- ata gaa ttc cat gca gaa aaa aaa caa acg taa cac caa c -3'OH

SEQ ID NO:2

5'HO- cgg ctg aga acg ttc aga ggt ttt aac gat ctg acc acc acc cgg g -3'OH

SEQ ID NO:3

5'HO- aaa acc tct gaa cgt tct cag ccg -3'OH

SEQ ID NO:4

5'HO- tat gga tcc tta tta cgg aga cag cag cca acc agc -3'OH

SEQ ID NO:5

1 GAATTCCATG CAGAAAAAAA ACAAACGTAA CACCAACCGT CGTCCGCAGG

51 ACGTTAAATT CCCGGGTGGT GGTCAGATCG TTAAAACCTC TGAACGTTCT

101 CAGCCGCGTG GGCGTCGTCA GCCGATCCCG AAAGCTCGTC GTCCGGAAGG

151 TCGTACCTGG GCTCAGCCGG GTTACCCGTG GCCGCTGTAC GGTAACGAAG

201 GTTGCGGTTG GGCAGGTTGG CTGCTGTCTC CGTAATAAGG ATCC

SEQ ID NO:6

1 MQKKNKRNTN RRPQDVKFPG GGQIVKTSER SQPRGRRQPI PKARRPEGRT

51 WAQPGYPWPL YGNEGCGWAG WLLSP**

SEQ ID NO:7

1 GAATTCCATG CAGAAAAAAA AGAAACGTAA CACCAACCGT CGTCCGCAGG

51 ACGTTAAATT CCCGGGTGGT GGTCAGATCG TTGGTCTGCT GCCGCGTCGT

101 GGTCCGCGTC TGGGTCGTAA AACCTCTGAA CGTTCTCAGC CGCGTGGGCG

151 TCGTCAGCCG ATCCCGAAAG CTCGTCGTCC GGAAGGTCGT ACCTGGGCTC

201 AGCCGGGTTA CCCGTGGCCG CTGTACGGTA ACGAAGGTTG CGGTTGGGCT

251 GGTTGGCTGC TGTCTCCGTA ATAAGGATCC

SEQ ID NO:8

1 MQKKNKRNTN RRPQDVKFPG GGQIVGLLPR RGPRLGRKTS ERSQPRGRRQ

51 PIPKARRPEG RTWAQPGYPW PLYGNEGCGW AGWLLSP**

SEQ ID NO:9

5'HO- acc cag acg cgg acc acg acg cgg cag cag acc aac gat ctg acc acc acc c -3'OH

SEQ ID NO:10

5'HO- ccg cgt cgt ggt ccg cgt ctg ggt cgt aaa acc tct gaa cgt tct cag -3'OH

SEQ ID NO:11

1 GAATTCCATG CAGAAAAAAA ACAAACGTAA CACCAACCGT CGTCCGCAGG

51 ACGTTAAATT CCCGGGTGGT GGTCAGATCG TTGGTGGTGT TTACGTTCTG

101 CCGCGTCGTG GTCCGCGTCT GGGTGTTCTG GCTACGCGTA AAACCTCTGA

151 ACGTTCTCAG CCGCGTGGGC GTCGTCAGCC GATCCCGAAA GCTCGTCGTC

201 CGGAAGGTCG TACCTGGGCT CAGCCGGGTT ACCCGTGGCC GCTGTACGGT

251 AACGAAGGTT GCGGTTGGGC TGGTTGGCTG CTGTCTCCGT AATAAGGATC

301 C

SEQ ID NO:12

1 MQKKNKRNTN RRPQDVKFPG GGQIVGGVYV LPRRGPRLGV LATRKTSERS

51 QPRGRRQPIP KARRPEGRTW AQPGYPWPLY GNEGCGWAGW LLSP**

SEQ ID NO:13

5'HO- acc cag acg cgg acc acg acg cgg cag aac gta aac acc acc aac -3'OH

SEQ ID NO:14

5'HO- ccg cgt cgt ggt ccg cgt ctg ggt gtt ctg gct acg cgt aaa acc -3'OH

SEQ ID NO:15

1 GAATTCCATG CAGAAAAAAA ACAAACGTAA CACCAACCGT CGTCCGCAGG

-continued

```
 51 ACGTTAAATT CCCGGGTGGT GGTCAGATCG TTGGTGGTGT TTACCTGCTN

101 CCGCGTCGTG GTCCGCGTCT GGGTGTTCGT GCTACGCGTA AAACCTCTGA

151 ACGTTCTCAG CCGCGTGGGC GTCGTCAGCC GATCCGAAAG CTCGTCGTCC

201 GGAAGGTCGT ACCTGGGCTC AGCCGGGTTA CCCGTGGCCG CTGTACGGTA

251 ACGAAGGTTG CGGTTGGGCT GGTTGGCTGC TGTCTCCGTA ATAAGGATCC
```

SEQ ID NO:16

```
  1 MQKKNKRNTN RRPQDVKFPG GGQIVGGVYL LPRRGPRLGV RATRKTSERS

 51 QPRGRRQPIP KARRPEGRTW AQPGYPWPLY GNEGCGWAGW LLSP**
```

EXAMPLE IV

Preparation of Microparticles

Microparticles, coated with several monoclonal antibodies, were prepared by coating several separate populations of microparticles with HCV monoclonal antibodies which recognize different regions within the HCV core protein. Similarly, microparticles were coated with recombinant antigens cloned from the NS3 and NS4 regions of HCV. The peptide used for microparticle coating was from the core region of HCV.

Microparticles for Antibody Assay:

The following recombinant proteins and peptide were used to coat the microparticles for antibody assay.

A. Preparation of Recombinant Proteins:

i. HCV HC43 antigen HCV. HC43 recombinant antigen was obtained from Chiron Corporation, Emeryville, Calif. It contained amino acid sequence 1–150 (corresponding to the core protein) and 1192–1457 (corresponding to amino acid residues within NS3) of HCV-1 (amino acid sequence available from GenBank®, as described hereinabove)

ii HCV C-100 antigen. HCV C-100 recombinant antigen was obtained from Chiron Corporation, Emeryville, Calif. It contained amino acid sequence 1569–1961 (corresponding to amino acid residues within NS4) of HCV-1 (available from GenBank®, as described hereinabove)

iii HCV NS5 antigen. HCV NS5 recombinant antigen was obtained from Chiron Corporation, Emeryville, Calif. It contained amino acid sequence 2054–2995 of HCV (available from GenBank®, described hereinabove)

iv HCV NS3 NS4 *E Coli* construct CKS-33c-BCD antigen. HCV HC31 recombinant antigen was obtained from Chiron corporation, Emerville, Calif. It contained amino acid Sequence 1192–1457 of HCV , and amino acid sequence 1676–1931 of the NS4 region. In addition it consists of 239 amino acids of CKS. (available from GenBank®, described hereinabove).

A1. Preparation of R-antigen Coated Microaprticles

i. Preparation of HCV HC43/C100 Microparticles

Microparticles coated with both HC43 and c-100 were prepared in the following manner. Briefly, a 500 µl aliquot of microparticles (10% weight/volume, 0.7–0.9 micron, available from Seradyn, Indianapolis, Ind.) was mixed with 962 µl of a coating buffer (Phosphate buffer, pH 5.0 with Tween-20) for approximately 1 minute at room temperature. Then, 154 µl of an HCV C100-3 antigen solution (0.65 mg/ml) and 308 ul of an HC43 antigen solution (650 µg/ml) were added to the microparticle solution, mixed and tumbled for 16 hours at room temperature. The microparticles were pelleted at 12,000×g for 10 minutes in an Eppendorf microfuge. The suspension was removed and the microparticles were washed with wash buffer (Phosphate, NaCl, dithiothreitol-DTT, EDTA, sodium dodecyl sulfate-SDS, pH 6.5) and heat stressed at 56 Degrees C for 20 hours. The microparticles were then resuspended in 2.5 ml of microparticle diluent (Phosphate Buffer, pH 6.5, EDTA, DTT, NaCl and SDS, Sucrose, azide) at a final concentration of 2.0%.

ii. Preparation of HCV NSS Microparticles

530 µl of an HCV NS5 coating buffer (Carbonate, pH 10, SDS) and 200 ul of a 10% weight/volume 0.7–0.9 micron microparticles (available from Seradyn, Indianapolis, Ind.) were added to 270 µl of the HCV NS5 antigen solution (concentration of 650 µg/ml). The microparticles were mixed and tumbled for 16 hours at room temperature. The microparticles were pelleted at 12,000×g for 10 minutes in an Eppendorf microfuge. The suspension was removed and the microparticles were washed with wash buffer (Phosphate, NaCl, DTT, EDTA, SDS, pH 6.5) and heat stressed at 56 Degrees C for 20 hours. The washed microparticles were then resuspended in 2.5 ml of microparticle diluent (Phosphate Buffer, pH 6.5, EDTA, DTT, NaCl and SDS, Sucrose, azide) at a final concentration of 0.4%.

iii. Preparation of HCV NS3 NS4 *E. Coli* Construct CKS-33c-BCD Microparticles

A 100 ul aliquot of microparticles (10% weight/volume, 0.7–0.9 micron, available from Seradyn, Indianapolis, Ind.) was mixed with 452 µl of coating buffer (Phosphate buffer, pH 5.0 with Tween-20) for approximately 10 minutes at room temperature. Then, 200 µg of CKS-33C-BCD Ag was added and mixed for 16 hours at room temperature.

The microparticles were pelleted at 12,000×g for 10 minutes in an Eppendorf microfuge. The prepared microparticles were washed with wash buffer (DTT, EDTA, SDS in PBS, pH 6.5). The supernatant was removed and the microparticles were resuspended in 1 ml of microparticle diluent (Phosphate Buffer, pH 6.5, EDTA, DTT, NaCl, Sucrose and SDS, Sucrose).

iv. Blending of HCV HC43/C100 and HCV NS5 Microparticles

220 µl of HCV HC43/C100 microparticles prepared as described in Example (IV)(A1)(i) and 330 µl of HCV NS5 microparticles prepared as described in Example (IV)(Al)(ii) were mixed together. This mixture was incubated at room temperature for 15 minutes and diluted to 50 ml in microparticle diluent. (Phosphate Buffer, pH 6.5, EDTA, DTT, NaCl, Sucrose and SDS, Sucrose).

v. Preparation of Biotinylated Core Peptide

HCV core peptide aa 11–28 was biotinylated at N-terminus during synthesis using an automated peptide synthesizer with ≧90% purity.

vi. Preparation of Streptavidin Coated Microparticles

A four ml aliquot of carboxylated microparticles (10% weight/volume, 0.227 micron, Seradyn, Indianapolis, Ind) was mixed with 2486 ul of coupling buffer (MES (2-(N-morpholino) ethanesulfonic acid) pH 6.7) for 10 minutes at room temperature. Then, 114.4 µl of EDAC solution (10 mg/ml in coupling buffer) was added to the microparticle solution and mixed for 15 minutes at room temperature. Subsequently, 1 ml of Streptavidin solution (1 mg/ml in PBS) was added to the activated microparticles and tumbled for 16 hours at room temperature. The prepared microparticles were then pelleted at 12,000×g for 3 minutes in an Eppendorf microfuge. The supernatant was removed and the microparticles were resuspended in 4 ml of PBS. The centrifugation process was repeated one more time and finally microparticles were stored in 4 ml of PBS to yield a final concentration of approximately 1%.

vii. Preparation of Core Peptide Coated Microparticles

To 1 ml of coated microparticles from Example (IV) (A1) (vi) was added 375 µl HCV Core peptide from Example (IV)(A1)(v) and 11–28 aa at 1 mg/ml in PBS buffer. The mixture was then incubated for 2 hours at room temperature. The prepared microparticles were washed with wash buffer (DTT, EDTA, SDS in PBS, pH 6.5) and the microparticles were resuspended in 1 ml of microparticle diluent (Calf Bovine Serum, Horse IgG, TWEEN 20, BSA, Casein, EDTA, Sucrose and Proclin, pH 6.5) yielding 1% solids final concentration.

Microparticles for the Antigen Assay:

B. Preparation of Monoclonal Antibodies

The methods for generating monoclonal antibodies were presented in U.S. Pat. No. 5,753,430. Briefly, *E coli* derived recombinant antigens encoded by HCV sequences, designated as pHCV34 (HCV-core, a.a. 1–150), was employed as immunogen for antibodies to core. Detailed information on the cloning of pHCV34 is disclosed in U.S. patent application Ser. No. 07/572,822, incorporated herein by reference. The protein was prepared for immunization with appropriate adjuvants after purification, as would be performed by those skilled in the art.

BALB/c mice were injected intraperitoneally with 15 ug of purified pHCV34 with 15 ug each of Trehalose dimycolate (TDM) and M. phlei in a buffer immulsion prepared according to the manufacturer's instructions. Subsequent immunizations were performed on day 14, 28 and 42. Mice were bled on days 21 and 49 and the immune response was studied by enzyme-linked immunosorbent assay utilizing pHCV34 coated on polystyrene beads, as detailed in U.S. Pat. No. 5,753,430.

Upon demonstration of specific anti-HCV antibody present at reasonable titers in the sera of immunized mice, mice were bosted with 40 ug of pHCV34 antigen. The mice were sacricied and their spleens were removed; the white cells were mixed and fused with SP2/0 cells. The cell mixture was cultured in Iscoe's Modified Dulbecco's Medium (IMDM) supplemented with 20% fetal calf serum, and the hybridoma cells were selected by using a hypoxanthine and thymidine medium. Hybridoma cell lines were established, and all monoclonal antibodies specific for antibodies to core were prepared from ascite fluids of the mice and were purified by chromatography on a protein-A column (Pharmacia, Uppsala, Sweden). The epitopes of the monoclonal antibodies were analyzed by an ELISA test described in example I.

B1. Preparation of Monoclonal Antibody Coated Microparticles for Antigen Assay

i. Preparation of HCV C11-14 Microparticles

Briefly, a 1 ml aliquot of carboxylated microparticles (10% weight/volume, 0.227 micron, available from Seradyn, Indianapolis, Ind.) was mixed with 9 ml of coupling buffer (MES (2-(N-morpholino) ethanesulfonic acid) pH 6.7) for approximately 10 minutes at room temperature. Then 150 µl of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 10 mg/ml in coupling buffer, Sigma Chemical Company) was added to the microparticle solution and mixed for 15 minutes at room temperature. 1822 µl of C11-14 monoclonal antibody solution (2.13 mg/ml) was added to the activated microparticles, mixed and tumbled for 16 hours at room temperature. The microparticles were then pelleted at 12,000×g for 3 minutes in an Eppendorf microfuge. The supernatant was removed and the microparticles were washed with microparticle wash buffer (Phosphate Buffer Saline-PBS, Tween 20, pH 7.2), followed by washing with microparticle coating buffer (Tris Buffer Saline-TBS, Bovine Serum Albumin-BSA, pH 7.2) and final washing with microparticle final exchange buffer (PBS, Tween 20, pH 7.2). The Microparticles were resuspended in 5 ml of final exchange buffer and heat stressed at 45 Degrees C for 72 hours. After heat stress, 5 ml of microparticle diluent (Calf Bovine Serum, Horse IgG, Tween 20, BSA, Casein, Ethylene diamine tetraacetic acid-EDTA, Sucrose and Proclin, pH 6.5) was added to give a final concentration of approximately 1.0%.

ii. Preparation of HCV A5 (14-635-225) Mab Microparticles

A similar procedure as mentioned in Example (IV) (B1)(i) was used, except that instead of C11-14 Mab, A5 14-635-225)Mab was used for coating on microparticles.

iii Preparation of HCV C11-3 Microparticles

6.6 µl of 1N HCl was added to 300 µl (1.45 mg/ml) of C11-3 monoclonal antibody to bring the pH to 2.5. The monoclonal was then incubated at this pH for 5 minutes. The pH was then brought to 6.5 by adding 50 mM MES buffer. A 100 µl aliquot of carboxylated microparticles (10% weight/volume, 0.227 micron, Seradyn, Indianapolis, Ind.) was then mixed with 333 µl of coupling buffer (MES, pH 6.7) for 10 minutes at room temperature. Then 15 µl of EDAC solution (10 mg/ml in coupling buffer) was added to the microparticle solution and mixed for 5 minutes at room temperature. 552 µl of pH shocked C11-3 monoclonal antibody solution (0.725 mg/ml) was added to the activated microparticle, mixed and tumbled for 16 hours at room temperature. The microparticles were then pelleted at 12,000×g for 3 minutes in an Eppendorf microfuge. The supernatant was removed, and the microparticles were washed with microparticle wash buffer (Phosphated Buffered Saline (PBS), Tween 20, pH 7.2), followed by a wash with microparticle coating buffer (Tris Buffered Saline (TBS), Bovine Serum Albumin (BSA), pH 7.2) and a final wash with microparticle final exchange buffer (PBS, Tween 20, pH 7.2). The microparticles were resuspended in 0.5 ml of final exchange buffer and heat stressed at 45 degrees Celsius for 72 hours. After heat stress, 0.5 ml of microparticle diluent (Calf Bovine Serum, Horse IgG, Tween 20, BSA, Casein, ethylene diamine tetraacetic acid (EDTA), Sucrose, and ProClin, pH 6.5) was added to give a final concentration of approximately 1.0%.

iv. Blending of HCV C11-14 and C11-3 Microparticles

36 µl of HCV C11-3 microparticles (1% solid) prepared as described in Example (IV)(B1)(iii) and 84 µl HCV C11-14 microparticles (1% solids) prepared as described in Example (IV)(B1)(i) were mixed with 880 µl microparticle diluent (Calf Bovine Serum Horse IgG, Tween 20, BSA, Casein, EDTA, Sucrose, and Proclin, pH 6.5).

C. Preparation of Microparticles for Combo Assay:

For the dual assay, two separate PRISM channels were used, one for the HCV antibody assay and one for the HCV antigen assay. For the combo assay both the antibody and antigen assays were performed on a single channel where the reagents for both antigen and antibody assay was combined in one kit.

i . Blending of C11-14 mAb Coated Microparticles with Core Antigen (Peptide) Coated Microparticles and HCV HC33 Antigen Coated Microparticles

350 µl of core peptide coated microparticles (1% solids stock) prepared as in Example (IV)(A1)(vii) and 700 µl of HCV NS3 NS4 *E. coli* Construct CKS-33C-BCD Ag coated microparticles (1% solids stock) prepared as described in Example (IV)(A1)(iii) and 319 µl HCV C11-14 microparticles (1.0099% solids stock) prepared as described in Example (IV)(B1)(i) were mixed with 5631 µl microparticle diluent (calf Bovine Serum, Horse IgG, Tween 20, BSA, Casein, EDTA, Sucrose and Proclin, pH 6.5).

EXAMPLE V

Preparation of Acridinium-Labeled Conjugates

A. Conjugate for Antibody Assay:

For the antibody assay either mouse anti human IgG directly labeled with acridinium or a pre-complex of biotinylated anti-human F $(ab')_2$ and acridinium anti biotin conjugate was used.

(i) Pre-complex of Biotinylated Anti-human $F(ab')_2$ and Acridinium Anti-biotin Conjugate:

The labeled anti-biotin antibody was prepared as disclosed in U.S. Pat. No. 5,705,330. The pre-complex of biotinylated Anti-human $F(ab')_2$ and acridinium anti-biotin conjugate were also prepared as disclosed in U.S. Pat. No. 5,705,330.

(ii) Acridinium Labeled Mouse Anti-human IgG:

53.6 µl of conjugation buffer (CB) containing sodium phosphate, NaCl, 3-(3-chlolamidopropyl)-dimethylammonio-1-propane-sulfonate (CHAPS, Sigma Chemical Company, Saint Louis, Mo.), pH 8.0 and 7.2 µl of N-hydroxysuccinimide ester of 10-(3-sulfopropyl)-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide (4 mg/ml in dimethyl formamide) was added to 131 µl of Mouse anti-Human IgG (4.59 mg/ml) and 601 µl of PBS at room temperature. The reaction mixture was mixed with a rotator for 20 minutes at room temperature. The reaction was quenched by loading the reaction mixture onto the HPLC. This was applied to a 300×7.8 mm Bio-Sil SEC-250 gel filtration column (Bio-Rad, Richmond, Calif.) which had been equilibrated with buffer containing CHAPS, NaCl and sodium phosphate, pH 6.3. The column was eluted at 1.0 ml/min with the same buffer using a Beckman 421A controller equipped with a model 114M pump. Fractions of 1 ml were collected and the absorbance determined at 280 nm and 370 nm with a Beckman DU-7 spectrophotometer. The extent of acridinium incorporation was calculated as described in U.S. Pat. No. 5,705,330. The acridinium to IgG ratio (mole/mole) obtained was approximately 2.5. The conjugate was stored at 4 degrees C.

B. Conjugate for Antigen Assay:

Acridinylation of c11-10 Conjugate

A similar procedure as mentioned in Example (V) (A) (ii) was used except for the following changes. 700 ul of conjugate buffer, 300 ul (1 mg/ml) of C11-10 Mab, and 2.9 ul (4 mg/ml) of acridinium derivative were mixed for 10 minutes at room temperature. The acridinium to IgG ratio (mole/mole) obtained was approximately 2.0. The conjugate was stored at 4 degrees C.

C. Conjugate for Combo Assay:

Blending of Acridinylated Mouse Anti-Human IgG and Acridinylated C11-10 Mab Conjugate:

Fourteen ul of Acridinylated Mouse anti-Human IgG (1 ug/ml) was mixed with 390 ul of Acridinylated C11-10 Mab (1.79 ug/ml) conjugate yielding 2 ng/ml Mouse anti-Human IgG with 100 ng/ml C11-10, incubated for 2 hours and filtered before use. Preparation of Mouse anti-Human IgG and Acridinylated C11-10 Mab conjugate are described in Example (V)(A)(ii) and (V)(B) respectively.

EXAMPLE VI

Detection of HCV Core Protein by Monoclonal Antibodies

Since a large number of anti-HCV core monoclonal antibodies were available for use in developing an antigen detection assay, it was necessary to determine which combination of monoclonal monoclonals would provide the greatest sensitivity. Because the number of combinations possible when using more than one monoclonal on the solid phase (i.e. for capture) and in the liquid phase (i.e. detection) is extremely large, a simplified "screening" method was used to identify the best performing pair of monoclonals. It was assumed that once the most sensitive pair was identified, other monoclonals could be added to improve assay sensitivity, if necessary.

In order to identify the best pairs, therefore, monoclonal antibodies were coated onto microparticles or conjugated with acridinium as described in Example IV and V. Screening assays used monoclonal antibody coated microparticles (0.40 µm diameter) at a working concentration of 0.09–0.15% solids and conjugated monoclonals at a working concentration of 100–125 ng/ml. For all experiments, the same positive and negative control plasma were used (0.1 ml for each assay). The positive control serum was from an HCV-infected individual who tested negative for HCV antibodies but whose plasma had an HCV RNA titer of 19,000,000 copies per ml. The negative control plasma was from a normal blood donor who was negative for HCV antibodies and RNA. Assays were performed using the instrumentation and operation methods as described in Example VIII.

Table IV shows the mean signal-to-negative (S/N) values obtained upon testing the various pairs of monoclonal antibodies for their ability to detect HCV core antigen in the positive control human plasma (nd: not determined). From this data, it is apparent that some pairs of monoclonalantibodies exhibit greater sensitivity than others and that the sensitivity was dependent upon the proper configuration of the assay. For example, when monoclonal antibody A05 was used as the capture reagent and C11-10 was used as the detection reagent, the resulting S/N value was 150.0, however, when the opposite configuration was used, the resulting S/N value was only 6.8.

TABLE IV

Detection of HCV Core Antigen in Human Plasma by Various Pairs of Anti-core Monoclonal Antibodies

		CONJUGATE													
	Mab	107-35-54	14-635-225	14-726-217	13-975-157	13-959-270	14-178-125	14-886-216	110-81-17	14-945-218	C11-3	C11-7	C11-10	C11-14	C11-15
		Mab													
MICRO-PARTICLE		A04	A05	A06	A08	A09	A13	A14	A15	A218	C11-3	C11-7	C11-10	C11-14	C11-15
14-1708-403	A01	0.6	nd	1.3	2.5	1.0	1.3	5.2	1.0	1.4	2.4	0.9	53.0	1.9	3.5
14-153-462	A02	nd	nd	1.4	nd	0.9	1.5	1.5	nd	2.4	0.6	1.0	22.0	1.8	0.2
14-1269-281	A03	0.8	nd	1.3	2.4	1.1	1.3	7.4	0.9	1.4	2.2	2.9	101.6	3.0	6.0
107-35-54	A04	nd	nd	0.9	nd	nd	nd	nd	nd	nd	1.7	1.2	7.6	12.9	1.3
14-635-225	A05	1.2	nd	1.5	2.9	0.9	1.0	8.4	1.0	1.5	4.2	2.1	150.0	1.9	4.0
14-726-217	A06	nd	nd	0.6	nd	1.6	1.8	2.5	nd	2.7	1.7	2.4	9.5	3.8	0.7
13-975-157	A08	nd	nd	0.7	nd	nd	nd	nd	nd	nd	1.4	0.8	4.6	1.6	3.2
13-959-270	A09	nd	nd	1.6	nd	nd	nd	nd	nd	nd	1.5	1.2	2.4	1.5	1.0
14-1705-255	A10	nd	nd	0.7	nd	1.3	1.7	1.2	nd	2.5	1.6	1.3	13.6	4.5	1.2
14-188-104	A11	nd	nd	0.8	nd	1.0	1.2	2.8	nd	1.2	2.8	0.7	16.5	1.8	2.4
14-1287-252	A12	nd	nd	0.9	nd	nd	nd	nd	nd	nd	2.8	1.9	6.3	2.4	6.1
14-886-216	A14	nd	nd	0.9	nd	nd	nd	nd	nd	nd	3.2	3.1	3.0	4.2	2.1
110-81-17	A15	nd	nd	1.5	nd	nd	nd	nd	nd	nd	1.0	1.9	6.3	2.4	2.3
14-947-104	A16	0.5	nd	1.1	6.1	1.5	1.3	6.1	1.1	1.1	1.6	1.4	69.0	4.7	1.2
C11-3	C11-3	0.6	4.7	nd	nd	1.1	2.1	1.0	nd	0.9	2.0	1.1	11.1	4.6	3.6
C11-7	C11-7	1.2	3.9	nd	nd	1.1	0.8	3.6	nd	1.3	2.5	1.8	6.2	3.4	2.0
C11-10	C11-10	0.6	6.8	nd	nd	0.9	1.4	1.1	nd	1.0	8.2	1.9	4.3	4.6	2.8
C11-14	C11-14	0.8	2.0	1.5	nd	0.8	1.1	13.4	nd	1.7	7.9	0.9	208.0	2.2	14.4
C11-15	C11-15	1.6	4.6	nd	nd	1.2	1.4	1.3	nd	1.5	5.1	1.5	4.9	3.8	5.7

EXAMPLE VII

HCV Core Antigen Assay Sample Diluent Buffer

The HCV core antigen assay for PRISM, as described in Example XI, utilizes a sample diluent buffer (SDB) for dilution of the human serum or plasma sample to be tested. The monoclonal-antibody coated microparticles are then added to form a reaction mixture. It is possible that the sensitivity and specificity of the antigen detection assay is affected by the composition of the SDB, in terms of the ingredients and their concentration.

It was hypothesized that, since HCV is believed to be an enveloped virus, it would be necessary to include detergent (surfactant) in the SDB to remove the lipid envelope, thereby exposing the core protein to solution. In addition, it was surmised that addition of chaotropic salts to the SDB might aid in dissolution of the nucleocapsid complex which could enhance detectability of core antigen.

To investigate the possible effects of SDB composition on the HCV core antigen assay sensitivity, a series of buffers was prepared and tested in an HCV core antigen assay comprised of monoclonal antibody C11-7 or C11-14 coated microparticles (as stated in the table legends) and acridinium labeled monoclonal antibody C11-10 conjugate. The simplest SDB used (also referred to as basal buffer), in terms of number of components, consisted of 0.1 M potassium phosphate, pH 7.2, 10 mM EDTA. This is the buffer to which detergents and salts were added. The performance of the SDBs was determined by examining their effect on the signal-to-negative (S/N) ratio obtained upon testing of a positive control human plasma from an individual who tested negative for HCV antibodies but whose plasma had an HCV RNA titer of 19,000,000 copies per ml. The negative control plasma was from a normal blood donor who was negative for HCV antibodies and RNA. Screening assays used coated microparticles at a working concentration of 0.09–0.15% solids and C11-10 conjugate at a working concentration of 100–125 ng/ml. For all experiments, the same positive and negative control plasma was used (0.1 ml for each assay). Assays were performed using the instrumentation and operation methods as described in Example VIII.

As shown in Table V, the S/N value obtained varies greatly depending upon the detergent added to the sample diluent buffer and its concentration. Addition of the zwitterionic surfactant SB-12 (lauryl sulfobetaine) gave the highest S/N values. In addition, as shown in Table VI, the highest S/N values were again seen with SB-12 when compared to other zwitterionic detergents of the same class but with different alkyl chain lengths. Titration of the amount of SB-12 added to the basal buffer in the presence of 0.5% or 2% Triton X-100 is shown in Table VII. Increasing the SB-12 concentration over 6% diminished S/N values obtained in the core antigen assay significantly.

Further experiments examined the effect of the addition of salts or different combinations of zwitterionic or nonionic detergents to the sensitivity of the core antigen assay. Results presented in Tables VIII and X suggest a marginal effect on S/N is observed when KCl is substituted for NaCl, the same is true for the addition of urea. The sample diluent buffers containing SB-16 (palmityl sulfobetaine) appear to exhibit enhanced S/N values. The effect of urea was examined by including increasing concentrations in one of the SDBs that gave reasonably high S/N values compared to the others in a previous experiment (Table X). In this particular buffer, addition of urea to a final concentration of 2.0–2.5 M appears to have increased S/N values most significantly.

The effect on S/N values by the addition of various proteins or serum from nonhuman sources to a sample diluent buffer was also examined (Table XI). The inclusion of bovine serum albumin, with or without other proteinacious components, only marginally increased the S/N values obtained upon testing of the HCV positive control serum. In

contrast, some combinations of protein or sera actually decreased the S/N value relative to that observed for the protein-free buffer.

TABLE V

Effect of Detergent on HCV Core Antigen Detection

Detergent/Additive (to basal buffer)	Acronym	S/N @ 0.5%	S/N @ 2%
Dodecyldimethyl-3-amonio-propane sulfonate	SB-12	2.4	7.9
1-dodecylpyridinium chloride	DPC	0.1	6.9
Sodium dodecylsulfate	SDS	2.7	5.0
Cholamidopropyldimethylamonio propanesulfonate	CHAPS	1.0	4.7
3a,7a,12a-Trihydroxy-5b-cholanic acid	Cholate	1.2	2.2
t-Octylphenoxypolyethoxyethanol	Triton X-100	2.0	1.8
Carboxymethyltrimethylammonium	Betaine	1.9	1.5
Taurocholic acid	TCA	1.1	1.3
Dodecyltrimethylammonium bromide	DTAB	0.2	1.1
Mixture of steroids, polysacc., detergents	Saponin	3.1	0.9
N-Dodecanoyl-N-methylglycine (N-lauroyl sarcosine)	NLS	1.6	0.2
Cetyltrimethylammonium bromide	CTAB	1.6	nd
Tetradecyltrimethylammonium bromide	TDTAB	1.6	nd

Assay format: Anti-HCV core monoclonal C11-7 coated microparticles (0.40 um) and C11-10 conjugate.
nd: not determined.

TABLE VI

Detergent/Additive (in basal buffer)	Acronym	S/N @ 0.5%	S/N @ 2%
(3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate	CHAPSO	2.9	11.5
N-dodecyl-N,N-(dimethylammonio)butyrate	DDMAB	1.9	nd
N-dodecyl-N,N-(dimethylammonio)undecanoate	DDMAU	4.1	9.4
N,N-dimethyldodecylamine-N-oxide	LDAO	3.8	1.8
N-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	SB-8	2.5	3.8
N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	SB-10	3.0	5.2
N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	SB-12	16.0	38.3
N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	SB-14	4.5	0.8
N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	SB-16, 0.125%	5.0	nd

Assay format: Anti-HCV core monoclonal C11-14 coated microparticles (0.40 um) and C11-10 conjugate.
nd: not determined.

TABLE VII

Detergent/Additive (in basal buffer)	S/N with 0.5% Triton X100	S/N with 2% Triton X100
2% SB-12	13.3	14.8
4% SB-12	14.3	16.6
6% SB-12	10.7	15.3
8% SB-12	0.8	0.91

Assay format: Anti-HCV core monoclonal C11-14 coated microparticles (0.40 um) and C11-10 conjugate.

TABLE VIII

SDB Components and Final Concentration

Nacl	SB-12	SB-16	CTAB	Triton X-100	Urea	Exp 1 S/N	Exp 2 S/N
(no buffer added)						3.85	nd
Water						3.41	4.7
0.5 M						2.84	6.7
0.5 M				1.80%		32.17	34.2
0.5 M	2%					93.4	78.2
0.5 M	2%				2.5 M	106.3	82.9
0.5 M	2%		0.10%			97.1	67.4
0.5 M	2%		0.10%		2.5 M	93.9	86.3
0.5 M	2%		0.10%	1.80%		98.8	79.1
0.5 M	2%		0.10%	1.80%	2.5 M	84.8	90.5
0.5 M		2%			2.5 M	105.2	92.9
0.5 M		2%	0.10%			106.8	108.1
0.5 M		2%	0.10%		2.5 M	142.2	102.3
0.5 M		2%	0.10%	1.80%		115.1	101.6
0.5 M		2%	0.10%	1.80%	2.5 M	nd	103.0

All SDBs were prepared in basal buffer containing 0.1 M potassium phosphate, pH 7.2, 10 mM EDTA. Assay format: Anti-HCV core monoclonal C11-14 coated microparticles (0.227 um) and C11-10 conjugate.

TABLE IX

SDB Components and Final Concentration

KCl	SB-12	SB-16	CTAB	Triton X-100	Urea	S/N
0.5 M	2%					48.6
0.5 M	2%				2.5 M	86.8
0.5 M	2%		0.10%			52.8
0.5 M	2%		0.10%		2.5 M	77.6
0.5 M	2%		0.10%	1.80%		83.6
0.5 M	2%		0.10%	1.80%	2.5 M	106.8
0.5 M		2%			2.5 M	122.6
0.5 M		2%	0.10%		2.5 M	136
0.5 M		2%	0.10%	1.80%		127.9
0.5 M		2%	0.10%	1.80%	2.5 M	113.7

All SDBs were prepared in basal buffer containing 0.1 M potassium phosphate, pH 7.2, 10 mM EDTA. Assay format: Anti-HCV core monoclonal C11-14 coated microparticles (0.227 um) and C11-10 conjugate.

TABLE X

Effect of Urea on Antigen Assay Sensitivity

HCV positive control plasma dilution factor	S/N values at various urea concentrations 0.0 M	0.5 M	1.0 M	1.5 M	2.0 M	2.5 M	3.0 M	5.0 M
1:2	35.2	31.3	32.9	33.4	43.0	34.6	33.2	1.1
1:4	19.9	15.7	17.7	13.6	21.5	18.9	15.6	1.1
1:8	9.1	8.2	9.3	6.1	10.5	8.1	8.0	0.9
1:16	5.9	4.3	5.7	5.3	6.6	5.6	5.5	1.0
1:32	3.8	3.2	3.6	3.3	3.6	3.5	3.0	1.0

Urea was added to increasing final concentrations in the following buffer: 0.1 M potassium phosphate, pH 7.2, 10 mM EDTA, 0.5 M Nacl, 2.0% SB-16, 0.10 CTAB, 1.8% Triton X-100. HCV positive control plasma was diluted in negtive control plasma. Assay format: Anti-HCV core monoclonal C11-14 coated microparticles (0.227 um) and C11-10 conjugate.

TABLE IX

Effect of Protein or Serum on Antigen Assay Sensitivity

Component(s) Added (final conc., w/v)y	S/N PC 1:2	S/N PC 1:16
No additions	69.3	9.6
1% BSA, 2% mouse serum	73.7	10.3
1% BSA, 0.1% casein	70.5	10.7
1% BSA	70.2	10.3
3% horse serum	68.2	9.6
2% mouse serum	65.6	10.9
0.1% casein	51.7	8.1
2% mouse serum, 0.1% casein	50.9	7.9
1% BSA, 3% horse serum	50.6	8.8
0.1% casein, 3% horse serum	40.7	6.9
2% mouse serum, 3% horse serum	34.3	4.9

Protein or sera were added to buffer containing 100 mM potassium phosphate, pH 7.2, 10 mM EDTA, 0.5 M NaCl, 2% SB-16, 1.10% CTAB, 1.8% Triton X-100, 2.5 M urea. Positive control plasma (PC) was diluted 1:2 or 1:16 in negative control plasma.

EXAMPLE VIII

PRISM HCV Ab, PRISM HCV Ag, and PRISM HCV Ab/Ag Combo Assays

The PRISM antibody assay is described in U.S. Pat. No. 5,705,330, incorporated herein by reference. and the PRISM antigen and antibody assays are described in Shah and Stewart, The Immunoassay Handbook, second edition, edited by David Wild, p 297–303 (2001), also incorporated herein by reference.

With respect to the present invention, the following procedures were utilized:

HCV Ab Assay:

Assay Format is Provided in FIG. **1**.

Generally, at station 1, 50 µl of control or sample, 50 µl of specimen diluent buffer (SDB, Phosphate buffer, pH 7.0 containing Tween 20, newborn calf serum, NaCl, superoxide dismutase (SOD), *E. coli* lysate and azide), and 50 µl of HCV antigen coated microparticles [(prepared as described in Example (IV)(A1)(iv) above] were dispensed into each incubation well and assay timing was started. These were mixed by mutual diffusion of each into the other without external agitation or shaking to form a reaction mixture. At station 4, the reaction mixture was transferred to a detection well which contained a fibrous matrix and washed twice with 300 µl of transfer wash (TW, containing borate buffer, pH 7.0, with NaCl, Tween −20, Glycerol and Proclin 300). After 18 minutes of incubation at room temperature, 50 µl of a pre-complexed biotinylated $F(ab')^2$/acridinium labeled anti-biotin, (biotinylated $F(ab')^2$ fragment of goat anti-human IgG and acridinium labeled anti-biotin antibody), was dispensed into the matrix of the detection well at station 5. The well was incubated for 23 minutes at 37 degrees C., and the fibrous matrix containing the reaction mixture was washed three times with 100 µl of FW, containing MES (2-[N-morpholino] ethanesulfonic acid), pH 5.7, with NaCl and Proclin 300 at station 8. At station 9, as in all of the assays described below, a chemiluminescence (CL) signal was generated by addition of an alkaline hydrogen peroxide solution, and the photons were measured by a photo multiplier tube. The amount of light emitted is proportional to the amount of antibody in the sample. The presence or absence of antibody in the sample is determined by comparing the number of photons collected from the sample to a cutoff (S/CO) value determined from a calibration performed in the batch. The results are expressed as S/CO (signal to cutoff) in Table XII below. The cutoff value is calculated by the sum of product of the average chemiluminescence counts of the positive control (n=4) times 0.55 plus the average chemiluminescence counts of the negative control (n=4).

Sensitivity: The seroconversion sensitivity was 100% as compared to the HCV RNA data provided in vendor certificate of analysis data for the selected seroconversion panels tested. Data is summarized in Table XII.

Specificity: Based on repeat reactive rates, the specificity of the HCV Ab assay was >99% with the population tested (Table XV).

HCV Ag Assay:

Assay Format is Provided in FIG. **2**.

Mab C11-14/Mab C11-10 Pair:

Generally, at station 1, 100 µl of control or sample, 50 µl of specimen diluent buffer (SDB, Sodium phosphate, EDTA, Triton X-100, Urea and sodium azide), and 50 µl of HCV Mab coated microparticles (prepared as described in Example (IV)(B1)(i)) were dispensed into each incubation well and the assay timing was started. These were mixed by mutual diffusion of each into the other without external agitation or shaking to form a reaction mixture. At station 4, the reaction mixture was transferred to a detection well which contained a fibrous matrix and washed twice with 300 µl of transfer wash (TW) (MES, NaCl, Triton X-100, PEG, Antifoam, Proclin 300, pH 5.6) after 18 minutes of incubation at room temperature. At station 5, 50 µl of acridinylated C11-10 Mab conjugate (as mentioned in Example (V)(B)) was dispensed into the matrix of the detection well. The contents of the well were incubated for 23 minutes, and the fibrous matrix containing the reaction mixture was washed one time with 200 µl of final wash (FW) (Tris buffer with LiCl and LDS) followed by three times with 100 ul of FW. The CL signal was triggered and measured at station 9. The results are expressed as S/CO (signal to cutoff) in Table XII. The cutoff value is 2.2 times the average chemiluminescence count of the negative control (n=5).

Sensitivity: Two groups of commercially available seroconversion panels containing serially collected samples from individuals who developed antibodies to HCV were tested in the prototype PRISM HCV antigen test and in the PRISM HCV antibody test. For the first group of seroconversion samples, the first available bleed date was negative for HCV RNA. In subsequent bleed dates, HCV RNA was detected for one or more bleed dates, followed in all cases by detection of antibodies to HCV. For the second group of seroconversion panels, the first bleed date was already positive for HCV RNA; antibodies to HCV were detected in subsequent bleed dates. For the two groups seroconversion sensitivity was 98.5% as compared to data obtained by HCV RNA testing. The PRISM HCV Ag detected 67/68 HCV RNA positive bleeds as reactive. Data is summarized in Table XIII. These data indicate that HCV Ag testing detects HCV infection in individuals who have not yet mounted an antibody response.

Specificity: Based on repeat reactive rates, the specificity of the HCV Ag assay was >99% with the population tested (Table XVI).

HCV Ag Assay: Mab A5 (14-635-225)/Mab C11-10 Pair:

An assay procedure, similar to that mentioned for C11-14/C11-10, was used. The only difference was that the test used Abbott A5 (14-635-225) Mab coated microparticles instead of Mab C11-14 coated microparticles.

Sensitivity: A total of 4 seroconversion panels were evaluated and sensitivity was compared with the data generated using C11-14/C11-10 pair. Both these pairs detected the same number of positive bleeds. Sensitivity data for A5 (14-635-225)/C11-10 pair is summarized in Table XIV.

Specificity: Based on repeat reactive rates, the specificity of the HCV Ag assay was 100% with the mini population (n=100) tested (Table XVII).

HCV Ag Assay: Mab C11-14 and C11-3/Mab C11-10 Pair:

An assay procedure, similar to that mentioned for C11-14/C11-10, was used. The only difference was the use of C11-14 and C11-3 blended microparticles (Example (IV) (B1)(iv)) instead of Mab C11-14 coated microparticles.

Sensitivity: The performance of this pair was assessed by comparing the S/N ratio against panels consisting of recalcified human plasma positive for HCV Core antigen (termed "PC") and a human plasma negative for HCV antigens and antibodies (termed "NC") (Table XVIII). The S/N was determined by the formula:

$$S/N = \text{Average of } PC / \text{Average of } NC$$

The average chemiluminescence counts of four specimen was used to determine each average.

PRISM HCV Ag/Ab Combo Assay:

Two different formats (i.e., Dual Combo assay and Real combo assay) were evaluated on the PRISM HCV Ag/Ab Assay as follows:

Dual combo assay: The HCV Ag/Ab dual combo assay is run simultaneously on PRISM using two different channels. A total of six channels in PRISM are used simultaneously to run several assays (HIV, HBcore, HBsAg, HTLV, and HCVAb) five of which are currently in use, while one channel remains open for new markers (e.g. HCV Ag assay) or can be reserved in case one of the channels become problematic. Thus, by using one channel for an HCV Ag assay and five other channels for five other assays, a reserve channel is not available for use.

The PRISM HCV Ab and PRISM HCV Ag assays were performed individually. The results from both assays were combined to produce single, final report.

Real combo assay: The PRISM HCV Ag and HCV Ab assays were combined and performed as a single assay in one of the PRISM channels.

PRISM HCV Ag/Ab Dual Combo Assay:

Sensitivity: The seroconversion sensitivity of the HCV Ab/Ag Dual Combo assay was 98.5%. Data is summarized in Table XII.

Specificity: Based on repeat reactive rates, the specificity of the HCV Ab/Ag Dual Combo assay was >99% with the population tested (Table XIX).

PRISM HCV Ag/Ab Real Combo Assay:

Assay format is provided in FIG. **3**. The 2 step PRISM HCV Combo assay was performed as mentioned above for the HCV Ab or Ag assay with the following changes: At station 1, 100 ul of control or sample, 50 ul of specimen diluent buffer (Phosphate buffer, pH 7.0 containing Tween 20, newborn calf serum, NaCl, Tween-20, superoxide dismutase (SOD), *E. coli* lysate and azide), and 50 ul of HCV antigen and Mab blended microparticles (prepared as described in Example (IV)(C)(i) above) were dispensed into each incubation well and the assay timing was started. At station 4, the reaction mixture was transferred to a detection well which contained a fibrous matrix and washed twice with 300 ul of transfer wash (MES, NaCl, Triton X-100, PEG, Antifoam, Proclin 300, pH 5.6). After 18 minutes of incubation at 37 degree C., 50 ul of blended conjugate acridinylated C11-10 and Acridinylated Mouse anti-Human IgG (prepared as described in Example (V)(C)) was dispensed into the matrix of the detection well at station #5. The well was incubated for 23 minutes, and the fibrous matrix containing the reaction mixture was washed three times with 100 ul of final wash (Tris buffer with LiCl and LDS). The CL signal was triggered and measured at station 9. The results are expressed as S/CO (signal to cutoff) in Table II below. The cutoff value is 2.2 times the average chemiluminescence count of the negative control (n=3).

Sensitivity: The seroconversion sensitivity was 95.8% as compared to PCR data. The PRISM HCV Ag/Ab Real Combo assay detected 23/24 positive bleeds as reactive. Data is summarized in Table XIII.

Specificity: Based on repeat reactive rates the specificity of HCV Ag/Ab Real Combo assay was 100% with the population tested (Table XX):

TABLE XII

Genotype	Ag earlier than Ab by	Day Number	HCV RNA Test Results	HCV Ab S/CO	HCV Ag S/CO	HCV Ab/Ag Dual Combo
1a	38 Days	0	–	0.09	0.49	NEG
		24	+	0.11	10.63	POS
		27	+	0.10	43.77	POS
		31	+	0.11	72.92	POS
		62	+	5.19	44.41	POS
		64	+	5.22	69.55	POS
		69	+	5.91	12.92	POS
		71	+	6.29	7.09	POS
1a	20 Days	0	+	0.10	107.78	POS
		3	+	0.11	97.81	POS
		10	+	0.15	63.52	POS
		20	+	1.57	Not Tested	POS
1b	18 Days	0	+	0.09	76.61	POS
		4	+	0.09	56.09	POS
		7	+	0.08	39.63	POS
		13	+	0.34	32.14	POS
		18	+	1.53	14.93	POS
		21	+	3.20	19.97	POS
		164	Not Tested	5.86	0.61	POS
2b	14 Days	0	+	0.10	8.41	POS
		2	+	0.40	23.62	POS
		7	+	2.62	14.09	POS
		9	+	3.09	16.67	POS
		14	+	3.99	4.74	POS
1a	42 Days	0	–	0.09	0.76	NEG
		2	–	0.08	0.47	NEG
		7	–	0.09	0.41	NEG
		9	–	0.09	0.40	NEG
		15	–	0.08	0.42	NEG
		17	–	0.08	0.49	NEG
		22	–	0.07	0.49	NEG
		24	–	0.08	0.45	NEG
		29	–	0.09	0.52	NEG
		31	–	0.08	0.51	NEG
		36	–	0.08	0.57	NEG
		38	–	0.09	0.51	NEG
		43	–	0.08	0.52	NEG
		45	–	0.09	0.43	NEG
		50	–	0.09	0.69	NEG
		52	–	0.08	0.52	NEG
		57	–	0.09	0.49	NEG
		64	–	0.09	0.90	NEG

TABLE XII-continued

Geno-type	Ag earlier than Ab by	Day Number	HCV RNA Test Results	HCV Ab S/CO	HCV Ag S/CO	HCV Ab/Ag Dual Combo
		67	–	0.08	0.52	NEG
		74	–	0.09	0.51	NEG
		79	–	0.08	0.52	NEG
		84	–	0.09	0.40	NEG
		105	–	0.09	0.45	NEG
		108	–	0.08	0.66	NEG
		112	–	0.09	0.57	NEG
		119	–	0.09	0.45	NEG
		121	–	0.09	0.42	NEG
		140	+	0.12	10.65	POS
		143	+	0.09	3.81	POS
		147	+	0.10	9.30	POS
		150	+	0.09	34.08	POS
		154	+	0.09	58.01	POS
		157	+	0.09	80.90	POS
		161	+	0.08	107.11	POS
		164	+	0.09	114.41	POS
		168	+	0.09	93.29	POS
		171	+	0.10	89.06	POS
		182	+	1.83	63.62	POS
		186	+	4.39	68.72	POS
		189	+	5.20	119.62	POS
1a	21 Days	0	+	0.08	63.86	POS
		4	+	0.07	50.76	POS
		17	+	0.09	73.66	POS
		21	+	1.02	45.58	POS
		25	+	4.03	60.94	POS
		29	+	5.08	47.38	POS
1a	37 Days	0	+	0.08	33.66	POS
		2	+	0.06	30.83	POS
		7	+	0.08	30.10	POS
		9	+	0.07	39.66	POS
		26	+	0.07	25.51	POS
		32	+	0.12	15.29	POS
		37	+	2.43	15.51	POS
		41	+	3.36	3.10	POS
1a	28 Days	0	+	0.09	67.75	POS
		2	+	0.09	87.93	POS
		10	+	0.10	36.53	POS
		12	+	0.10	60.67	POS
		19	+	0.10	39.62	POS
		21	+	0.11	26.25	POS
		28	+	2.78	9.94	POS
		30	+	4.00	17.02	POS
		35	+	4.71	15.26	POS
		37	Not Tested	4.84	13.02	POS
1a	25 Days	0	+	0.15	4.73	POS
		2	+	0.40	6.63	POS
		8	+	0.16	7.48	POS
		10	+	0.11	5.20	POS
		16	+	0.17	7.60	POS
		18	+	0.11	7.58	POS
		23	+	0.64	8.66	POS
		25	+	2.11	9.58	POS
		30	+	2.76	6.21	POS
		32	+	3.39	7.84	POS
		49	+	6.12	1.83	POS
		53	+	6.13	1.93	POS
		56	+	6.34	1.63	POS
1a	28 Days	0	–	0.13	1.27	POS
		2	–	0.23	0.52	NEG
		8	–	0.09	0.50	NEG
		11	+	0.10	0.54	NEG
		15	+	0.10	1.92	POS
		18	+	0.11	1.90	POS
		28	+	0.12	2.42	POS
		30	+	0.10	7.04	POS
		35	+	0.14	6.01	POS
		37	+	0.98	13.68	POS
		43	+	4.74	10.07	POS
		46	+	5.27	4.91	POS
1a	13 Days	0	+	0.10	1.82	POS
		3	+	0.10	1.72	POS
		5	+	0.15	1.35	POS
		11	+	0.97	1.70	POS
		13	+	1.26	3.63	POS
		19	–	3.70	2.94	POS
		25	–	4.89	2.43	POS
		27	–	5.20	1.61	POS
		32	–	5.61	1.35	POS
		35	–	5.86	1.30	POS
		41	–	6.11	0.88	POS
		45	–	5.69	0.67	POS
		48	–	5.95	1.94	POS
1a	23 Days	0	–	0.09	0.45	NEG
		2	–	0.08	0.45	NEG
		17	+	0.07	20.06	POS
		19	+	0.09	45.84	POS
		24	+	0.09	81.03	POS
		26	+	0.07	63.30	POS
		36	+	0.31	74.78	POS
		40	+	4.03	49.53	POS
1a	33 Days	0	–	0.09	0.54	NEG
		3	–	0.08	0.50	NEG
		7	–	0.08	0.58	NEG
		12	–	0.09	0.54	NEG
		14	–	0.09	0.56	NEG
		19	–	0.10	0.53	NEG
		25	–	0.10	0.51	NEG
		28	–	0.09	0.50	NEG
		32	–	0.09	0.50	NEG
		35	–	0.10	0.35	NEG
		39	–	0.09	0.53	NEG
		45	+	0.10	9.43	POS
		47	+	0.11	42.00	POS
		52	+	0.11	28.05	POS
		56	+	0.11	25.63	POS
		60	+	0.09	78.15	POS
		73	+	0.18	9.54	POS
		78	+	1.83	5.34	POS
		80	+	2.13	3.40	POS
1a	32 Days	0	–	0.46	0.54	NEG
		22	–	0.42	0.53	NEG
		24	–	0.43	0.47	NEG
		42	+	0.46	8.79	POS
		46	+	0.44	22.26	POS
		74	+	4.22	19.82	POS
		76	+	4.50	23.78	POS
3a	141 Days	0	+	0.12	2.81	POS
		4	+	0.49	1.95	POS
		11	+	2.48	1.41	POS
		13	+	2.54	1.41	POS
		44	+	4.29	0.43	POS
		46	+	4.68	0.43	POS

* S/CO values ≧1.00 are considered reactive.

TABLE XIII

Geno-type	Ag earlier than Ab by	Day Number	HCV RNA Test Results	HCV Ab S/CO	HCV Ag S/CO	HCV Ab/Ag real Combo
1a	38 Days	0	–	0.09	0.49	0.38
		24	+	0.11	10.63	1.60
		27	+	0.10	43.77	5.32
		31	+	0.11	72.92	11.04
		62	+	5.19	44.41	10.95
		64	+	5.22	69.55	10.16
		69	+	5.91	12.92	4.51
		71	+	6.29	7.09	3.68

TABLE XIII-continued

Geno-type	Ag earlier than Ab by	Day Number	HCV RNA Test Results	HCV Ab S/CO	HCV Ag S/CO	HCV Ab/Ag real Combo
1b	18 Days	0	+	0.09	76.61	14.09
		4	+	0.09	56.09	5.59
		7	+	0.08	39.63	4.75
		13	+	0.34	32.14	3.81
		18	+	1.53	14.93	4.16
		21	+	3.20	19.97	3.81
		164	Not Tested	5.86	0.61	2.72
1a	23 Days	0	–	0.09	0.45	0.31
		2	–	0.08	0.45	0.48
		17	+	0.07	20.06	1.36
		19	+	0.09	45.84	2.72
		24	+	0.09	81.03	4.57
		26	+	0.07	63.30	4.15
		36	+	0.31	74.78	6.90
		40	+	4.03	49.53	5.11
1a	32 Days	0	–	0.46	0.54	0.48
		22	–	0.42	0.53	0.31
		24	–	0.43	0.47	0.32
		42	+	0.46	8.79	0.99
		46	+	0.44	22.26	2.11
		74	+	4.22	19.82	3.99
		76	+	4.50	23.78	3.27

* S/CO values ≧1.00 are considered reactive.

TABLE XV

N Tested	989*
RR	9
RRR	0.91
N negative	980
Mean S/CO Neg Pop	0.12
SD	0.09
SD to CO	9.76

TABLE XVI

N Tested	989*
RR	1
RRR	0.10
N negative	988
Mean S/CO Neg Pop	0.44
SD	0.08
SD to CO	7.15

*Volunteer blood donors

TABLE XIV

Geno type	Ag earlier than Ab by	Day Number	HCV RNA Test Results	HCV Ab S/CO	HCV Ag c11-14/ c11-10 S/CO	HCV Ag A5 c11-10	HCV Ab/Ag Dual Combo
1a	38 Days	0	–	0.09	0.49	0.53	NEG
		24	+	0.11	10.63	6.47	POS
		27	+	0.10	43.77	29.30	POS
		31	+	0.11	72.92	64.97	POS
		62	+	5.19	44.41	37.22	POS
		64	+	5.22	69.55	39.55	POS
		69	+	5.91	12.92	7.54	POS
		71	+	6.29	7.09	4.42	POS
1b	18 Days	0	+	0.09	76.61	68.95	POS
		4	+	0.09	56.09	48.69	POS
		7	+	0.08	39.63	57.02	POS
		13	+	0.34	32.14	34.85	POS
		18	+	1.53	14.93	16.61	POS
		21	+	3.20	19.97	13.78	POS
		164	Not Tested	5.86	0.61	0.62	NEG
1a	23 Days	0	–	0.09	0.45	0.80	NEG
		2	–	0.08	0.45	0.43	NEG
		17	+	0.07	20.06	7.77	POS
		19	+	0.09	45.84	17.28	POS
		24	+	0.09	81.03	30.76	POS
		26	+	0.07	63.30	27.95	POS
		36	+	0.31	74.78	36.66	POS
		40	+	4.03	49.53	21.78	POS
1a	32 Days	0	–	0.46	0.54	0.45	NEG
		22	–	0.42	0.53	0.47	NEG
		24	–	0.43	0.47	0.53	NEG
		42	+	0.46	8.79	5.04	POS
		46	+	0.44	22.26	14.18	POS
		74	+	4.22	19.82	14.15	POS
		76	+	4.50	23.78	8.54	POS

* S/CO values ≧1.00 are considered reactive.

TABLE XVII

N Tested	100*
RR	0
RRR	0.00
N negative	100
Mean S/CO Neg Pop	0.56
	0.15
SD to CO	2.98

*Volunteer blood donor

TABLE XVIII

	C11-10 conjugate:		
Panel	C11-14 microparticle only	C11-3 microparticl only	C11-14 + C11-3 Blended microaprticle
PC S/N	213.7	87.8	179.4
NC	78.8	90	93.25
Counts			

TABLE XIX

N Tested	989*
RR	9
RRR	0.91
N negative	980
Mean S/CO Neg Pop	0.12
SD	0.09
SD to CO	9.76

TABLE XX

N Tested	92*
RR	0
RRR	0
N negative	92
Mean S/CO Neg Pop	0.49
SD	0.15
SD to CO	3.4

*Volunteer blood donors

SEQUENCE LISTING

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<220> FEATURE:
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 2

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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 3

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 4

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<210> SEQ ID NO 5
<211> LENGTH: 244
<212> TYPE: DNA
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 5

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gccgatcccg aaagctcgtc gtccggaagg tcgtacctgg gctcagccgg gttacccgtg 180

gccgctgtac ggtaacgaag gttgcggttg ggcaggttgg ctgctgtctc cgtaataagg 240

atcc 244

<210> SEQ ID NO 6
<211> LENGTH: 75
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 6

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

Lys Phe Pro Gly Gly Gly Gln Ile Val Lys Thr Ser Glu Arg Ser Gln
20 25 30

Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly
35 40 45

Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu
50 55 60

Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
65 70 75

<210> SEQ ID NO 7
<211> LENGTH: 280
<212> TYPE: DNA
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 7

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cccgggtggt ggtcagatcg ttggtctgct gccgcgtcgt ggtccgcgtc tgggtcgtaa 120

aacctctgaa cgttctcagc cgcgtgggcg tcgtcagccg atcccgaaag ctcgtcgtcc 180

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cggttgggct ggttggctgc tgtctccgta ataaggatcc 280

<210> SEQ ID NO 8
<211> LENGTH: 87
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 8

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

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

Pro Arg Leu Gly Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg
35 40 45

Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala
50 55 60

Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp
65 70 75 80

Ala Gly Trp Leu Leu Ser Pro
85

<210> SEQ ID NO 9
<211> LENGTH: 52
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 9

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<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 10

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<210> SEQ ID NO 11
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<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 11

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gggtgttctg gctacgcgta aaacctctga acgttctcag ccgcgtgggc gtcgtcagcc 180

gatcccgaaa gctcgtcgtc cggaaggtcg tacctgggct cagccgggtt acccgtggcc 240

gctgtacggt aacgaaggtt gcggttgggc tggttggctg ctgtctccgt aataaggatc 300

c 301

<210> SEQ ID NO 12
<211> LENGTH: 94
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 12

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

Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Val Leu Pro
20 25 30

Arg Arg Gly Pro Arg Leu Gly Val Leu Ala Thr Arg Lys Thr Ser Glu
35 40 45

-continued

Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg
50 55 60

Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr
65 70 75 80

Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
85 90

<210> SEQ ID NO 13
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 13

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<210> SEQ ID NO 14
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 14

ccgcgtcgtg gtccgcgtct gggtgttctg gctacgcgta aaacc 45

<210> SEQ ID NO 15
<211> LENGTH: 300
<212> TYPE: DNA
<213> ORGANISM: Hepatitis C Virus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (100)...(100)
<223> OTHER INFORMATION: n = a or g or c or t/u, unknown, or other at position 100

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gggtgttcgt gctacgcgta aaacctctga acgttctcag ccgcgtgggc gtcgtcagcc 180

gatccgaaag ctcgtcgtcc ggaaggtcgt acctgggctc agccgggtta cccgtggccg 240

ctgtacggta acgaaggttg cggttgggct ggttggctgc tgtctccgta ataaggatcc 300

<210> SEQ ID NO 16
<211> LENGTH: 94
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 16

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

Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu Pro
20 25 30

Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu
35 40 45

Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg
50 55 60

Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr
65 70 75 80

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Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
85 90

<210> SEQ ID NO 17
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 17

gatcgctcga attcctcg 18

<210> SEQ ID NO 18
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 18

ttctagcgag cttaaggagc 20

<210> SEQ ID NO 19
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 19

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

Arg Arg

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 20

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

Gly Gly

<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 21

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

Leu Pro

<210> SEQ ID NO 22
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 22

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

Arg Ala

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<210> SEQ ID NO 23
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 23

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

Gln Pro

<210> SEQ ID NO 24
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 24

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

Lys Ala

<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 25

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

Ala Gln

<210> SEQ ID NO 26
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 26

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

Gly Asn

<210> SEQ ID NO 27
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 27

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

Trp Leu Leu

<210> SEQ ID NO 28
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 28

Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser
1 5 10 15

Trp

-continued

<210> SEQ ID NO 29
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<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 29

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

Pro Arg Arg Arg Ser Arg Asn Leu Gly
20 25

<210> SEQ ID NO 30
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 30

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

Val Ile Asp Thr Leu Thr Cys Gly Phe
20 25

<210> SEQ ID NO 31
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 31

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

Asp Leu Met Gly Tyr Ile Pro Leu Val
20 25

<210> SEQ ID NO 32
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 32

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

Ala Pro Leu Gly Gly Ala Ala Arg Ala
20 25

<210> SEQ ID NO 33
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 33

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

Ala His Gly Val Arg Val Leu Glu Asp
20 25

<210> SEQ ID NO 34
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 34

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

-continued

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Val Asn Tyr Ala Thr Gly Asn Leu Pro
            20                  25


<210> SEQ ID NO 35
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 35

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

Phe Ser Ile Phe Leu Leu Ala
            20


<210> SEQ ID NO 36
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Hepatitis C Virus

<400> SEQUENCE: 36

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

Leu Thr Val Pro Ala Ser Ala
            20
```

The invention claimed is:

1. A method for simultaneously detecting the presence of at least one HCV antigen and at least one HCV antibody in a test sample comprising the steps of:

(a) contacting said test sample with: 1) at least one HCV antigen or portion thereof coated on a solid phase, for a time and under conditions sufficient for the formation of antibody/antigen complexes and 2) at least one HCV antibody coated on said solid phase, for a time and under conditions sufficient for the formation of antigen/antibody complexes, wherein said at least one antibody coated on said solid phase is C11-14;

(b) adding a conjugate to the resulting antibody/antigen complexes of (a)(1) for a time and under conditions sufficient to allow said conjugate to bind to the bound antibody in (a)(1), wherein said conjugate comprises a second antibody attached to a chemiluminescent compound capable of generating a detectable signal, and simultaneously adding a second conjugate to the resulting antigen/antibody complexes of (a)(2) for a time and under conditions sufficient to allow said conjugate to bind to the bound antigen in (a)(2), wherein said second conjugate comprises a third antibody attached to said chemiluminescent compound capable of generating a detectable signal, wherein said third antibody is C11-10; and

(c) detecting a single generated signal, presence of said signal indicating presence of said at least one HCV antigen, at least one HCV antibody, or both, in said test sample.

2. The method of claim **1** wherein said at least one HCV antigen coated on the solid phase is selected from the group consisting of core antigen, NS3, NS4, NS5, and portions thereof.

* * * * *

专利名称(译)	同时检测HCV抗原和HCV抗体的方法		
公开(公告)号	US7101683	公开(公告)日	2006-09-05
申请号	US09/891983	申请日	2001-06-26
[标]申请(专利权)人(译)	SHAH DINESHØ DAWSON乔治 MUERHOFF斯科特 蒋LILY 古铁雷斯罗宾 LEARY THOMAS P 德赛Suresh STEWART詹姆斯·L·		
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[标]发明人	SHAH DINESH O DAWSON GEORGE J MUERHOFF ANTHONY SCOTT JIANG XINGLI GUTIERREZ ROBIN A LEARY THOMAS P DESAI SURESH STEWART JAMES L		
发明人	SHAH, DINESH O. DAWSON, GEORGE J. MUERHOFF, ANTHONY SCOTT JIANG, XINGLI GUTIERREZ, ROBIN A. LEARY, THOMAS P. DESAI, SURESH STEWART, JAMES L.		
IPC分类号	C12Q1/00 C12Q1/70 G01N33/53 G01N33/543 C07K14/18 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N15/09 C12P21/04 G01N21/76 G01N33/00 G01N33/576		
CPC分类号	C07K14/005 G01N33/5767 C12N2770/24222 G01N2469/20 G01N2469/10		
其他公开文献	US20030108858A1		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及同时检测丙型肝炎病毒（HCV）抗原以及响应HCV抗原产生的抗体的方法。此外，本发明允许人们甚至在抗体发展之前检测感染早期，急性期的抗原，从而允许早期检测受感染的血液和血液制品，从而提高血液供应的安全性。

A PROTOTYPE ABBOTT PRISM™ HCV Ag/Ab COMBO
ASSAY FORMAT

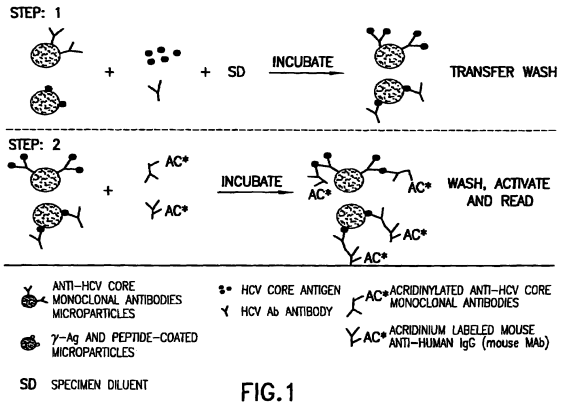

FIG.1