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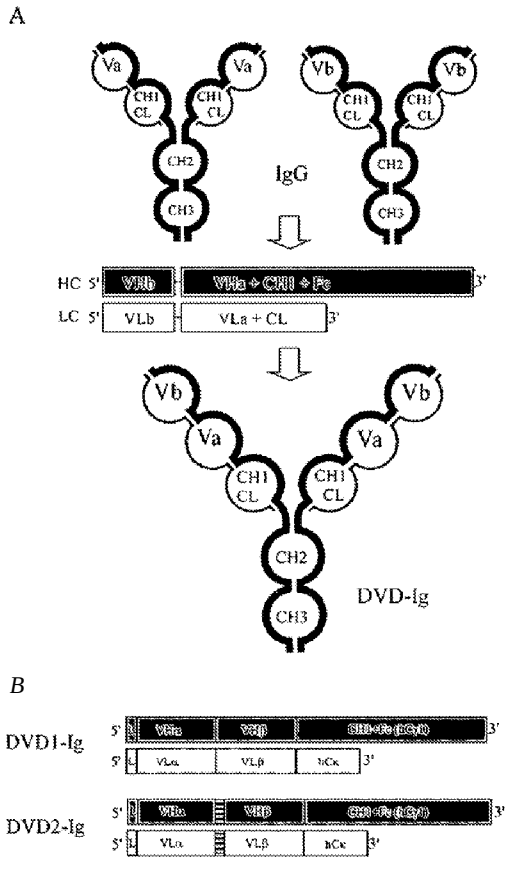
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(54) Title: MONOCLONAL ANTIBODIES AGAINST HEPATITIS C VIRUS CORE PROTEIN

(57) Abstract: The present disclosure relates to anti-HCV core protein monoclonal antibodies, methods of making them, and their uses in the prevention, diagnosis, and/or treatment of disease including HCV infections.

Figure 1



WO 2012/006500 A2

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MONOCLONAL ANTIBODIES AGAINST HEPATITIS C VIRUS CORE PROTEIN

Cross-Reference to Related Applications

This application claims priority to United States Provisional Application Serial No. 61/362,587 filed July 08, 2010, which is incorporated herein by reference in its entirety.

Field of the Invention

The present disclosure relates to binding proteins including monoclonal antibodies, and more particularly to nucleic acid sequences and encoded amino acid sequences of genes encoding humanized monoclonal antibodies directed against hepatitis C virus (HCV) core protein.

Background of the Invention

Recent epidemiological studies on HCV indicate that it infects more than 170 million people worldwide and that, in more than 50% of the cases, the infection is chronic. In the United States, approximately 4 million people are infected, and 30,000 new infections are estimated to occur annually (NIH Conference, Hepatology Suppl 1:2S (1997)). HCV causes cirrhosis, hepatocellular carcinoma, and is thus the primary reason for liver transplantation in the United States. HCV is responsible for 8,000-10,000 deaths annually in the United States.

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. During an eclipse phase period of approximately 10 days, viral presence is not evident (i.e., viral RNA cannot be detected), serum transaminase levels are within normal limits, and no evidence exists 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. Typically several weeks later, an increase in ALT levels is observed, indicating inflammation of the liver; antibodies are detected an average of about 70 days after exposure.

A preventive measure 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 (see, e.g., U.S. Patent No. 6,172,189). These tests may also be utilized in the clinical 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 (ClOO-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 la), 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. Transfusion 34:790-795 (1994)), 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 exposure.

Existing HCV antigen tests rely on detecting the presence of the HCV core antigen in serum or plasma. HCV core protein is a structural protein of HCV comprising the first 191 amino acids of the polyprotein and that forms the internal viral coat encapsidating the genomic RNA. Core protein has been shown to affect various intracellular events, including cell proliferation and apoptosis. However, the precise mechanisms of action are not fully understood. Core protein is also believed to play a major role in HCV-induced viral hepatitis. 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, SD161, 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 are 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 clinical 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.

Engineered proteins, such as multivalent antibodies capable of binding two or more antigen binding sites are known in the art. Such multispecific binding proteins can be

generated using cell fusion, chemical conjugation, or recombinant DNA techniques. For example, U.S. Patent No. 7,612,181 provides a novel family of binding proteins capable of binding two or more antigens with high affinity, which are called dual variable domain immunoglobulins (DVD-Ig™).

A need exists for improved binding proteins capable of binding two or more antigen binding sites, particularly those that can be used to detect HCV and treat HCV infection.

Summary of the Invention

The present disclosure provides the nucleotide and encoded amino acid sequences of the light and heavy chain variable regions of the genes encoding monoclonal antibodies directed against HCV core protein. Specifically, these monoclonals are known as AOT3, CI 1-3, CI 1-7, CI 1-9, and CI 1-14. The present disclosure also provides binding proteins capable of specific binding to HCV core protein.

Accordingly, in one aspect, the present disclosure provides a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the heavy chain of the monoclonal antibody comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9.

In another aspect, the present disclosure provides a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the light chain of the monoclonal antibody comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10.

In the above monoclonal antibodies, the variable region of the heavy chain may comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 1, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 2. The variable region of the heavy chain may comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 3, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 4. The variable region of the heavy chain may comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 5, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 6. The variable region of the heavy chain may comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 7, and the variable region of the light chain comprises an amino acid

sequence having at least 90% identity with SEQ ID NO: 8. The variable region of the heavy chain may comprise an amino acid having at least 90% identity with SEQ ID NO: 9, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 10. Any of the above monoclonal antibodies may a humanized or a chimeric antibody.

In another aspect, the present disclosure provides an isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes any of the above monoclonal antibodies. The nucleic acid molecule may comprise RNA or DNA.

In another aspect, the present disclosure provides an isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 11, 13, 15, 17 and 19.

In another aspect, the present disclosure provides an isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 12, 14, 16, 18 and 20.

In any of the above isolated nucleic acid molecules, the variable region of the heavy chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 11, and the variable region of the light chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 12. The variable region of the heavy chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 13, and the variable region of the light chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 14. The variable region of the heavy chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 15, and the variable region of the light chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 16. The variable region of the heavy chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID

NO: 17, and the variable region of the light chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 18. The variable region of the heavy chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 19, and the variable region of the light chain of the monoclonal antibody may be encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 20.

In another aspect, the present disclosure provides an isolated recombinant vector comprising any of the above nucleic acid molecules. Also provided is a host cell comprising such a recombinant vector.

In another aspect, the present disclosure provides a pharmaceutical or diagnostic composition comprising any one of the above monoclonal antibodies, and a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the present disclosure provides a method of reducing or eliminating human hepatitis C virus activity in a human subject, comprising administering to a subject in need thereof an effective amount of any of the above humanized monoclonal antibodies.

In another aspect, the present disclosure provides a method of detecting the presence of human hepatitis C virus in a sample, the method comprising contacting the sample with any of the above humanized monoclonal antibodies.

In another aspect, the present disclosure provides an immunoassay for determining the presence or amount of human hepatitis C virus in a sample, comprising: providing a reagent comprising any of the above humanized monoclonal antibodies; combining the monoclonal antibody reagent with the sample for a time sufficient for the monoclonal antibody reagent to bind to any human hepatitis C virus in the sample; and determining the presence or amount of human hepatitis C virus present in the sample based on specific binding of the monoclonal antibody reagent to the human hepatitis C virus core protein. In the immunoassay, the monoclonal antibody reagent may be immobilized or may be capable of being immobilized on a solid support. The monoclonal antibody reagent may be labeled with a detectable label. The method may further comprise: a) providing a reagent comprising a binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VDl-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CHI, and X2 is an Fc region; and two polypeptide chains comprise VDl-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light

chain variable domain, C is a light chain constant domain, XI is a linker with the proviso that it is not CHI, and X2 does not comprise an Fc region; and n is 0 or 1; wherein said four polypeptide chains of said binding protein form four functional antigen binding sites, wherein at least one heavy chain variable domain an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9, and at least one light chain variable domain comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10; and combining the binding protein reagent of (a) with the sample for a time sufficient for the binding protein reagent to bind to any human hepatitis C virus in the sample. The binding protein reagent may be immobilized or capable of being immobilized on a solid support.

In another aspect, the present disclosure provides an immunoassay device for detecting the presence or absence of human hepatitis C virus in a sample, the device comprising any of the above humanized monoclonal antibodies immobilized on a solid support.

In another aspect, the present disclosure provides a kit for detecting the presence or absence of human hepatitis C virus in a sample, the kit comprising any of the above humanized monoclonal antibodies, and instructions for determining whether the sample contains human hepatitis C virus. In the kit, the humanized monoclonal antibody may be immobilized on a solid support.

In another aspect, the present disclosure also provides a binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD_1-$(X_1)_n$-VD_2-C-$(X_2)_n$, wherein VDi is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, Xi is a linker with the proviso that it is not CHi, and X2 is an Fc region; and two polypeptide chains comprise VD_1-$p\zeta_1)_n$-VD_2-C-$p\zeta_2)_n$, wherein VDi is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, Xi is a linker with the proviso that it is not CHi, and X2 does not comprise an Fc region; and n is 0 or 1; wherein said four polypeptide chains of said binding protein form four functional antigen binding sites, wherein at least one heavy chain variable domain an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9, and at least one light chain variable domain comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10. The binding protein is for example capable of binding HCV core protein. The binding protein can be

capable of modulating a biological function of HCV core protein, or of neutralizing HCV core protein. The binding protein can comprise at least one amino acid sequence selected from the group consisting of SEQ ID NOS: 48-79. The binding protein may comprise at least one variable heavy chain domain and one variable light chain domain amino acid sequence selected from the group consisting of SEQ ID NOS: 48-79.

In another aspect, the present disclosure provides a pharmaceutical or diagnostic composition including any binding protein according to the present disclosure, and a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the present disclosure provides a method of reducing or eliminating human hepatitis C virus activity in a human subject, by administering to a subject in need thereof an effective amount of any binding protein according to the present disclosure.

In another aspect, the present disclosure provides a method of detecting the presence of human hepatitis C virus in a sample, by contacting the sample with any binding protein according to the present disclosure.

In another aspect, the present disclosure provides an immunoassay for determining the presence or amount of human hepatitis C virus in a sample, comprising: providing a reagent comprising a binding protein according to the present disclosure; combining the binding protein reagent with the sample for a time sufficient for the binding protein reagent to bind to any human hepatitis C virus in the sample; and determining the presence or amount of human hepatitis C virus present in the sample based on specific binding of the binding protein reagent to the human hepatitis C virus core protein. In the immunoassay, the binding protein reagent can be immobilized or is capable of being immobilized on a solid support. The binding protein reagent can be labeled with a detectable label. The method may further comprise providing a reagent comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, and combining the monoclonal antibody reagent with the sample for a time sufficient for the monoclonal antibody reagent to bind to any human hepatitis C virus in the sample. The monoclonal antibody reagent may be immobilized or capable of being immobilized on a solid support.

In another aspect, the present disclosure provides an immunoassay device for detecting the presence or absence of human hepatitis C virus in a sample, the device comprising: any binding protein according to the present disclosure immobilized on a solid support.

In another aspect, the present disclosure provides a kit for detecting the presence or absence of human hepatitis C virus in a sample, the kit comprising: any binding protein according to the present disclosure, and instructions for determining whether the sample contains human hepatitis C virus. In the kit, the binding protein can be immobilized on a solid support.

Brief Description of the Drawing

FIG. 1 is a schematic representation of Dual Variable Domain (DVD)-Ig constructs and shows the strategy for generation of a DVD-Ig from two parent antibodies.

Detailed Description of the Invention

The present disclosure provides novel monoclonal antibodies to the HCV core protein, and specifically the nucleotide and encoded amino acid sequences of the light and heavy chain variable regions of the genes encoding monoclonal antibodies directed against HCV core protein, compositions comprising them, methods of using them, immunoassays, and kits which contain these monoclonal antibodies. These monoclonal antibodies can be also used in engineered binding proteins such as DVD-Igs that bind to HCV core protein.

A. Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques as described herein are generally performed according to conventional methods well known in the art and as described in various general and more

specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present disclosure may be more readily understood, select terms are defined below.

The terms "polypeptide", "peptide" and "protein" as used interchangeably herein, refer to any polymeric chain of amino acids. The terms encompass native or artificial proteins, protein a fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric. Use of "polypeptide" herein is intended to encompass polypeptide and fragments and variants (including fragments of variants) thereof, unless otherwise contradicted by context. For an antigenic polypeptide, a fragment of polypeptide optionally contains at least one contiguous or nonlinear epitope of polypeptide. The precise boundaries of the at least one epitope fragment can be confirmed using ordinary skill in the art. The fragment comprises at least about 5 contiguous amino acids, such as at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, or at least about 20 contiguous amino acids. A variant of polypeptide is as described herein.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

"Biological activity" as used herein, refers to all inherent biological properties of the antigen. Biological properties include but are not limited to binding receptor; induction of cell proliferation, inhibiting cell growth, inductions of other cytokines, induction of apoptosis, and enzymatic activity.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CHI, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgAl and IgA2) or subclass.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an

immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter, et al. US Patent Nos 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions e.g., cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgGl and IgG3, mediate ADCC and CDC via binding to FcyRs and complement Clq, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. At least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber et al. Nature; 264: 415-20; Thies et al 1999 J Mol Biol; 293: 67-79.). Mutation of cysteine residues within the hinge regions to prevent heavy chain-heavy chain disulfide bonds will destabilize dimerization of CH3 domains. Residues responsible for CH3 dimerization have been identified (Dall'Acqua 1998 Biochemistry 37: 9266-73.). Therefore, it is possible to generate a monovalent half-Ig. Interestingly, these monovalent half Ig molecules have been found in nature for both IgG and IgA subclasses (Seligman 1978 Ann Immunol 129: 855-70; Biewenga et al 1983 Clin Exp Immunol 51: 395-400). The stoichiometry of FcRn: Ig Fc region has been determined to be 2:1 (West et al .2000 Biochemistry 39: 9698-708), and half Fc is sufficient for mediating FcRn binding (Kim et al 1994 Eur J Immunol; 24: 542-548.). Mutations to disrupt the dimerization of CH3 domain may not have greater adverse effect on its FcRn binding as the residues important for CH3 dimerization are located on the inner interface of CH3 b sheet structure, whereas the region responsible for FcRn binding is located on the outside interface of CH2-CH3 domains. However the half Ig molecule may have certain advantage in tissue penetration due to its smaller size than that of a regular antibody. At least one amino acid residue may be replaced in the constant region of the binding protein of the present disclosure, for example the Fc region, such that the dimerization of the heavy chains is disrupted, resulting in half DVD Ig molecules. The anti-inflammatory activity of IgG is completely dependent on sialylation of the N-linked glycan of

the IgG Fc fragment. The precise glycan requirements for anti-inflammatory activity has been determined, such that an appropriate IgGl Fc fragment can be created, thereby generating a fully recombinant, sialylated IgG 1 Fc with greatly enhanced potency (Anthony, R.M., et al. (2008) Science 320:373-376).

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHI domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHI domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al, (1989) Nature 341:544-546, Winter et al, PCT publication WO 90/05144 A l herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See, e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R.J., et al. (1994) Structure 2:1 121-1 123). Such antibody binding portions are known in the art. (See, e.g., Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5)). In addition, single chain antibodies also include "linear antibodies"

comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al, Protein Eng. 8(10): 1057-1062 (1995); and US Patent No. 5,641,870).

The term "multivalent binding protein" is used throughout this specification to denote a binding protein comprising two or more antigen binding sites. In one aspect, the multivalent binding protein is engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. Dual variable domain (DVD) binding proteins comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. DVDs as described herein can be monospecific, i.e., capable of one antigen such as HCV core protein, or multispecific, i.e. capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as DVD-Ig, and are described for example in U.S. Patent No. 7,612,181, the disclosure of which is herein incorporated by reference in its entirety. Each half of a DVD-Ig comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site.

The term "bispecific antibody", as used herein, refers to full-length antibodies that are generated by quadroma technology (see Milstein, C. and A. C. Cuello, Nature, 1983. 305(5934): p. 537-40), by chemical conjugation of two different mAbs (see Staerz, U. D., et al, Nature, 1985. 314(6012): p. 628-31), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (see Holliger, P., T. Prospero, and G. Winter, Proc Natl Acad Sci USA, 1993. 90(14): p. 6444-8.18), resulting in multiple different immunogloblin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is mono-valent for each antigen it binds to.

The term "dual-specific antibody", as used herein, refers to full-length antibodies that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT publication WO 02/02773). Accordingly a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bi-valent for each antigen it binds to.

A "functional antigen binding site" of a binding protein is one that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent antibody from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating antibody binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent antibody herein need not be quantitatively the same.

The term "linker" is used to denote polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R.J., et al. (1994) Structure 2:1 121-1123). Exemplary linkers include, but are not limited to, AKTTPKLEEGEFSEAR (SEQ ID NO: 21); AKTTPKLEEGEF SEARV (SEQ ID NO: 22); AKTTPKLGG (SEQ ID NO: 23); SAKTTPKLGG (SEQ ID NO: 24); SAKTTP (SEQ ID NO: 25); RADAAP (SEQ ID NO: 26); RADAAPTVS (SEQ ID NO: 27); RADAAAAGGPGS (SEQ ID NO: 28); RADAAAA$(G_4S)_4$ (SEQ ID NO: 29), SAKTTPKLEEGEF SEARV (SEQ ID NO: 30); ADAAP (SEQ ID NO: 31); ADAAPTVSIFPP (SEQ ID NO: 32); TVAAP (SEQ ID NO: 33); TVAAPSVFIFPP (SEQ ID NO: 34); QPKAAP (SEQ ID NO: 35); QPKAAPSVTLFPP (SEQ ID NO: 36); AKTTPP (SEQ ID NO: 37); AKTTPP SVTPLAP (SEQ ID NO: 38); AKTTAP (SEQ ID NO: 39); AKTTAP SVYPLAP (SEQ ID NO: 40); ASTKGP (SEQ ID NO: 41); ASTKGPSVFPLAP (SEQ ID NO: 42), GGGGSGGGGSGGGGS (SEQ ID NO: 43); GENKVEYAPALMALS (SEQ ID NO: 44); GPAKELTPLKEAKVS (SEQ ID NO: 45); GHEAAAVMQVQYPAS (SEQ ID NO: 46).

An "immunoglobulin constant domain" refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

The term "monoclonal antibody" or "mAb" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier

"monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the present disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R. (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W.E. (2002) Clin. Biochem. 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see, Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A. and Green L.L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al. (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. Such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) such that the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Exemplary affinity

matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. BidlTechnology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al. Gene 169:147- 155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al, J. Immunol. 154(7):3310-9 (1995); Hawkins et al, J. Mol. BioL 226:889-896 (1992) and selective mutation at selective mutagenesis positions, contact or hypermutation positions with an activity enhancing amino acid residue as described in US patent US 6,914,128 Bl.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and

typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In one aspect, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CHI, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e. hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al. (1971) Ann. NY Acad, Sci. 190:382-391 and, Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides

precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al, Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as LI, L2 and L3 or HI, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub- regions, and FRs represents two or more of the four sub- regions constituting a framework region.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non- lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al, Crit. Rev. Immunol. 22(3): 183-200 (2002); Marchalonis et al, Adv Exp Med Biol. 484:13-30 (2001)). One of the advantages

provided by various embodiments of the present disclosure stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CHI, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

As used herein, the term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds the antigen. In one aspect, the neutralizing binding protein binds the cytokine and reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85% or more.

The term "activity" includes activities such as the binding specificity and affinity of a DVD-Ig for two or more antigens.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side

chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Antibodies are said to "bind to the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jonsson, U., et al. (1993) Ann. Biol. Clin. 51:19-26; J5nsson, U., et al. (1991) Biotechniques 11:620-627; Johnsson, B., et al. (1995) J. Mol. Recognit. 8:125-131; and Johnnson, B., et al. (1991) Anal. Biochem. 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of a binding protein (e.g., an antibody) to the antigen to form the, e.g., antibody/antigen complex as is known in the art. The "K_{on}" also is known by the terms "association rate constant", or "k_a", as used interchangeably herein. This value indicating the binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen also is shown by the equation below:

$$\text{Antibody ("Ab")} + \text{Antigen ("Ag")} \rightarrow \text{Ab-Ag}$$

The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation, or "dissociation rate constant", of a binding protein (e.g., an antibody) from the, e.g., antibody/antigen complex as is known in the art. This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:

$$\text{Ab} + \text{Ag} \text{^} \text{Ab-Ag}$$

The term "K_D" as used herein, is intended to refer to the "equilibrium dissociation constant", and refers to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant **(k_{off})** by the association rate constant **(k_{on})**. The association rate constant, the dissociation rate constant and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho) can also be used.

"Label" and "detectable label" mean a moiety attached to a specific binding partner, such as an antibody or an analyte, e.g., to render the reaction between members of a specific binding pair, such as an antibody and an analyte, detectable, and the specific binding partner, e.g., antibody or analyte, so labeled is referred to as "detectably labeled." Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In one aspect, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); chromogens, fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety itself may not be detectably labeled but

may become detectable upon reaction with yet another moiety. Use of "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In one aspect , the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. When employed in the context of an immunoassay, the conjugate antibody may be a detectably labeled antibody used as the detection antibody.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (e.g., an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999)."

The terms "isolated polynucleotide" and "isolated nucleotide molecule" as used interchangeably herein mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" or "isolated nucleotide molecule" is found in nature, or does not occur in nature as part of a larger sequence. An "isolated polynucleotide" or

"isolated nucleotide molecule" may be operably linked to a polynucleotide that it is not linked to in nature.

The terms "regulate" and "modulate" as used interchangeably herein refer to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of a cytokine). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction. Correspondingly, the term "modulator," as used herein, is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of a cytokine). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in WOO 1/83525.

As used herein, the term "effective amount" refers to the amount of a therapy which is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

"Patient" and "subject" may be used interchangeably herein to refer to an animal, such as a mammal, including a primate (for example, a human, a monkey, and a chimpanzee), a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a whale), a bird (e.g., a duck or a goose), and a shark. Preferably, the patient or subject is a human, such as a human being treated or assessed for a disease, disorder or condition, a human at risk for a disease, disorder or condition, a human having a disease, disorder or condition, and/or human being treated for a disease, disorder or condition.

The term "sample", as used herein, is used in its broadest sense. A "biological sample", as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to,

humans, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, (e.g., whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

"Component," "components," and "at least one component," refer generally to a capture antibody, a detection or conjugate antibody, a control, a calibrator, a series of calibrators, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient urine, serum or plasma sample, in accordance with the methods described herein and other methods known in the art. Thus, in the context of the present disclosure, "at least one component," "component," and "components" can include a polypeptide or other analyte as above, such as a composition comprising an analyte such as polypeptide, which is optionally immobilized on a solid support, such as by binding to an anti-analyte (e.g., anti-polypeptide) antibody. Some components can be in solution or lyophilized for reconstitution for use in an assay.

"Control" refers to a composition known to not analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes).

"Predetermined cutoff and "predetermined level" refer generally to an assay cutoff value that is used to assess diagnostic/prognostic/therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (e.g., severity of disease, progression/nonprogression/improvement, etc.). While the present disclosure may provide exemplary predetermined levels, it is well-known that cutoff values may vary depending on the nature of the immunoassay (e.g., antibodies employed, etc.). It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other immunoassays based on this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, correlations as described herein (if any) should be generally applicable.

"Pretreatment reagent," e.g., lysis, precipitation and/or solubilization reagent, as used in a diagnostic assay as described herein is one that lyses any cells and/or solubilizes any analyte that is/are present in a test sample. Pretreatment is not necessary for all samples, as described further herein. Among other things, solubilizing the analyte (e.g., polypeptide of interest) may entail release of the analyte from any endogenous binding proteins present in the sample. A pretreatment reagent may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment reagent there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay.

"Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a predetermined positive/negative cutoff, can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction so as to comprise a "sensitivity panel."

"Risk" refers to the possibility or probability of a particular event occurring either presently or at some point in the future. "Risk stratification" refers to an array of known clinical risk factors that allows physicians to classify patients into a low, moderate, high or highest risk of developing a particular disease, disorder or condition.

"Specific" and "specificity" in the context of an interaction between members of a specific binding pair (e.g., an antigen (or fragment thereof) and an antibody (or antigenically reactive fragment thereof)) refer to the selective reactivity of the interaction. The phrase "specifically binds to" and analogous phrases refer to the ability of antibodies (or antigenically reactive fragments thereof) to bind specifically to analyte (or a fragment thereof) and not bind specifically to other entities.

"Specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like.

Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

B. Monoclonal Antibodies

The nucleotide (DNA) sequences and deduced protein sequences encoding the heavy and light chain variable domains of anti-HCV core monoclonal antibodies were obtained as follows: monoclonals AOT-3, CI 1-3, CI 1-7, CI 1-9 and CI 1-14 were produced by preparing hybridomas using spleen or lymphocytes removed from an immunized animal, by methods known to those skilled in the art such as those described in detail herein below and in, for example, Goding, J. W. 1983. Monoclonal Antibodies: Principles and Practice, Pladermic Press, Inc., NY, N.Y., pp. 56 97. Briefly, to produce a human-human hybridoma, a human lymphocyte donor is selected. A donor who is known as infected with HCV (where infection has been shown for example by the presence of anti-virus antibodies in the blood or by virus culture) may serve as a suitable lymphocyte donor. Lymphocytes can be isolated from a peripheral blood sample or spleen cells may be used if the donor is subject to splenectomy. Epstein-Barr virus (EBV) can be used to immortalize human lymphocytes or a human fusion partner can be used to produce human-human hybridomas. Primary in vitro immunization with peptides can also be used in the generation of human monoclonal antibodies. Antibodies secreted by the immortalized cells are screened to determine the clones that secrete antibodies of the desired specificity. For monoclonal anti-HCV core antibodies, the antibodies must bind to HCV core proteins respectively. Cells producing antibodies of the desired specificity are selected. Other methods for obtaining monoclonal antibodies can be used, as known in the art and described in detail herein below in the section describing parent antibodies for DVD bidning proteins. Example 1 below describes how the anti-HCV core monoclonals AOT-3, CI 1-3, CI 1-7, CI 1-9 and CI 1-14, were obtained and characterized following isolation of mRNA from hybridoma cells grown in cell culture. Deduced amino acid sequences of the heavy and light chain variable regions for the anti-HCV core monoclonal antibodies AOT-3, CI 1-3, CI 1-7, CI 1-9, and CI 1-14 are listed in Table 1.

Table 1: Deduced Amino Acid Seqs

Sequence Name	Sequence
HCV AOT3 VH (SEQ ID NO: 1)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS
HCV AOT3 VL (SEQ ID NO: 2)	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR
HCV Cll-3 VH (SEQ ID NO: 3)	QVQLQQPGAE LVKPGTSVKL SCKASGYTFT SYWMHWVKLR PGQGFEWIGE INPRYGDTSY NERFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYCSTDD YDRGFAYWGQ GTLVTVSA
HCV Cll-3 VL (SEQ ID NO: 4)	DVVMTQTPLT LSVTIGQPAS ISCKSSQSLL YSNGKNYLNW LFQRPGQSPK RLIYLVSKLD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCVQGTHFP YTFGGGTKLE IKR
HCV Cll-7 VH (SEQ ID NO: 5)	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS
HCV Cll-7 VL (SEQ ID NO: 6)	DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR
HCV Cll-9 VH (SEQ ID NO: 7)	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS

Sequence Name	Sequence
HCV Cll-9 VL (SEQ ID NO: 8)	QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR
HCV Cll-14 VH (SEQ ID NO: 9)	QVQLQQPGAE LVMPGASVKM SCKASGYTFT DYWMHWVKQR PGQGLEWIGA IDTSDSQTDY NQKYRGKATL TVDESASTAY MQLNSLTSED SAVYYCATIY YDYDGSFDQW GQGTTLTVSS
HCV Cll-14 VL (SEQ ID NO: 10)	DTVMTQSQKF MSTSVGDRVS ITCKASQNVR TVVAWYQQKP GQSPKALIYL ASNRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCLQ HWNYPLTFGG GTKLEIKR

The deduced amino acid sequences of each of SEQ ID NO: 1 through SEQ ID NO: 10 were deduced from the cDNAs encoding the heavy and light chain variable domains of anti-HCV core monoclonal antibodies. These nucleotide sequences are listed as SEQ ID NO: 11 through SEQ ID NO: 20 as shown in Table 2, wherein the amino acid sequence of SEQ ID NO: 1 (Table 1) is deduced from the nucleotide sequence shown in SEQ ID NO: 11, the amino acid sequence shown in SEQ ID NO: 2 (Table 1) is deduced from the nucleotide sequence shown in SEQ ID NO: 12, and so on. The deduced amino acid sequence of each of SEQ ID NO: 1 through SEQ ID NO: 10 starts at nucleotide 1 of the corresponding nucleic acid sequence shown in SEQ ID NOs: 11 through 20.

Table 2: Variable Region Nucleotide Sequences

Sequence Name	
HCV AOT3 VH (SEQ ID NO: 11)	CAGATCCAGT TGGTGCAGTC TGGACCTGAA CTGAAGAAGC CTGGAGAGAC AGTCAAGATC TCCTGCAAGG CTTCTGGTTA TTCCTTCACA GACTATTCAA TGCACTGGAT GAAACAGACT CCAGGAAAGG GTTTAAAGTG GATGGGCTGG ATAAACACTG AGACTGGTCA GCCAACATAT GCAGATGACT TCAAGGGACG GTTTGCCTTC TCTTTGGAAA CCTCTGCCAG CACTGCCTAT TTGCAGATCA ACAACCTCAA AAATGAGGAC ACGGCTACAT ATTTCTGTGC TCTCTGGGAC TACTGGGGCC AAGGCACCAC TCTCACAGTC TCCTCA
HCV AOT3 VL (SEQ ID NO: 12)	GATATTGTGA TGACGCAGGC TGCATTCTCC AATCCAGTCA CTCTTGGAAC ATCAGCTTCC ATCTCCTGCA GGTCTAGTAA GAGTCTCCTA CATAGTGATG GCATCACTTA TTTGTATTGG TTTCTGCAGA AGCCAGGCCA GTCTCCTCAG CTCCTGATTT ATCAGATGCC CAACGTTGCC TCAGGAGTCC CAGACAGGTT CAGTAGCAGT GGGTCAGGAA CTGATTTCAC TCTGAGAATC AGCAGAGTGG AGGCTGAGGA TGTGGGTGTT TATTACTGTG CTCAAAATCT AGAACTTCCT CCGACGTTCG GTGGAGGCAC CAAGCTGGAA ATCAGACGGG
HCV Cll-3 VH (SEQ ID NO: 13)	CAGGTCCAAC TCCAGCAGCC TGGGGCTGAA CTGGTGAAGC CTGGGACTTC AGTGAAGTTG TCCTGCAAGG CTTCTGGCTA CACCTTCACC AGCTACTGGA TGCACTGGGT GAAGCTGAGG CCTGGACAGG GCTTTGAGTG GATTGGAGAG ATTAATCCCC GATATGGTGA CACTAGTTAC AATGAGAGGT TCAAGAGAAA GGCCACACTG ACTGTAGACA AATCGTCCAG CACAGCCTAC ATGCAACTCA GCAGCCTGAC ATCTGAGGAC TCTGCGGTCT ATTACTGTTC AACCGATGAT TACGACAGGG GTTTTGCTTA CTGGGGCCAR GGGACTCTGG TCACTGTCTC TGCA

Sequence Name	
HCV Cll-3 VL (SEQ ID NO: 14)	GATGTTGTGA TGACCCAGAC TCCACTCACT TTGTCGGTTA CCATTGGCCA ACCAGCCTCT ATCTCTTGCA AGTCAAGTCA GAGCCTCTTA TATAGTAATG GAAAAAAC TA TTTGAATTGG TTATTTCAGA GGCCAGGCCA GTCTCCAAAG CGCCTAATCT ATCTGGTGTC TAAACTGGAC TCTGGAGTCC CTGACAGGTT CACTGGCAGT GGATCAGGAA CAGATTTTAC ACTGAAAATC AGCAGAGTGG AGGCTGAGGA TTTGGGAGTT TATTACTGCG TGCAAGGTAC ACATTTTCCT TACACGTTCG GAGGGGGGAC CAAGCTGGAA ATAAAACGG
HCV Cll-7 VH (SEQ ID NO: 15)	CAGCTGCAAC AGTTTGGAGC TGAGCTGGTG AAGCCTGGGG CTTCAGTGAA GATATCCTGC AAGGCTTCTG GCTACACATT CACTGAC TAC AACATGGACT GGGTGAAGCA GAGCCTTGGA AAGAGCCTTG AGTGGATTGG AGATATTAAT CCTAACGATG ATAGTACTAA CTACAACCAG AAGTTCAAGG GAAAGGCCAC ATTGACTGTA GACAAGTCCT CCAGAACAGC CTACATGGAG CTCCGCAGCC TGACATCTGA GGACACTGCA GTCTATTACT GTGCAAGAGA AGGTTACTGG TACTTCGATG TCTGGGGCGC AGGGACCACG GTCACCGTCT CCTCAG
HCV Cll-7 VL (SEQ ID NO: 16)	GATGTTTTGA TGACCCAAAC TCCACTCTCC CTGCCTGTCA GTCTTGGAGA TCAAGCCTCC ATCTCTTGCA GATCTAGTCA GAGCATTGTA TATAGTAATG GAAACACCTA TTTAGAATGG TACCTGCAGA AACCAGGCCA GTCTCCAAAG CTCCTGATCT ACAAAGTTTC CAAGCGATTT TCTGGGGTCC CAGACAGGTT CAGTGGCAGT GGATCAGGGA CAGATTTCAC ACTCAAGATC AGCAGAGTGG AGGCTGAGGA TCTGGGAGTT TATTACTGCT TTCAAGGTTC GCATGTTCCG TACACGTTCG GAGGGGGGAC CAAGCTGGAA ATAAAACGG
HCV Cll-9 VH (SEQ ID NO: 17)	CAGGTTACTC TGAAAGAGTC TGGCCCTGGG ATATTGCAGC CCTCCCAGAC CCTCAGTCTG ACTTGTTCTT TCTCTGGGTT TTCACTGAGC ACTTATGGTA TAGGAGTAGG CTGGATTCGT CAGCCTTCAG GGAAGGGTCT GGAGTGGCTG GCACACATTT GGTGGAATGA TGATAATTAC TATAACACAG CCCTGAAGAG CCGGCTCACA ATCTCCAAGG ATACCTCCAA CAACCAGGTA TTCCTCAAGA TCGCCAGTGT GGACACTGTA GATACTGCCA CATACTACTG TGTTCGAATA GGCGGTCCCG CTATGGAC TA CTGGGGTCAA GGAACCTCAG TCACCGTCTC CTCA
HCV Cll-9 VL (SEQ ID NO: 18)	CAAATTGTTC TCACCCCGTC TCCAGCAATC ATGTCTGCTT CTCCAGGGGA GAAGGTCACC ATAACCTGCA GTGCCAGCTC AAGTGTAAGT TACATGCACT GGTTCCAGCA GAAGCCAGGC ACTTCTCCCA AACTCTGGAT TTATAACACA TCCACCCTGG CTTCTGGAGT CCCTGCTCGC TTCAGTGGCA GTGGATCTGG GACCTCTTAC TCTCTCACAA TCAGCCGAAT GGAGGCTGAA GATGCTGCCA

Sequence Name	
	CTTATTACTG CCAGCAAAGG AGTAGTTACC CATACACGTT CGGAGGGGGG ACCAAGCTGG AAATAAAACG G
HCV Cll-14 VH (SEQ ID NO: 19)	CAGGTCCAAC TGCAGCAGCC TGGGGCTGAG CTTGTGATGC CTGGGGCTTC AGTGAAGATG TCCTGCAAGG CTTCTGGCTA CACATTCACT GACTACTGGA TGCACTGGGT GAAGCAGAGG CCTGGACAAG GCCTTGAGTG GATCGGAGCG ATTGATACTT CTGATAGTCA GACTGATTAC AATCAAAAGT ACAGGGGCAA GGCCACATTG ACTGTAGACG AATCCGCCAG CACAGCCTAC ATGCAACTCA ACAGCCTGAC ATCTGAGGAC TCTGCGGTCT ACTACTGTGC AACCATCTAC TATGATTACG ACGGGTCCTT TGACCAGTGG GGCCAAGGCA CCACTCTCAC AGTCTCCTCA
HCV Cll-14 VL (SEQ ID NO: 20)	GACACTGTGA TGACCCAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA CAGGGTCAGC ATCACCTGCA AGGCCAGTCA GAATGTTCGT ACTGTTGTAG CCTGGTATCA ACAGAAACCA GGGCAGTCTC CTAAAGCGCT GATTTACTTG GCATCCAACC GGCACACTGG AGTCCCTGAT CGCTTCACAG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATTAGCAA TGTGCAATCT GAAGACCTGG CAGATTATTT CTGTCTGCAA CATTGGAATT ATCCTCTCAC GTTCGGGGGG GGGACCAAAC TGGAAATAAA ACGG

The cDNA sequences set forth above represent exemplary embodiments of the disclosed cDNAs. Variations are contemplated in the cDNA sequences shown in SEQ ID NO: 11 through SEQ ID NO: 20. Such variations include those that will result in a nucleic acid sequence that is capable of directing production of analogs of the corresponding protein shown in SEQ ID NO: 1 through SEQ ID NO: 10. It will be understood that due to the degeneracy of the genetic code, many substitutions of nucleotides may be made that will lead to a DNA sequence that remains capable of directing production of the corresponding protein or its analogs. All such variant DNA sequences that are functionally equivalent to any of the sequences described herein, are encompassed by the present disclosure.

A variant of any of the binding proteins described herein means a protein (or polypeptide) that differs from a given protein (e.g., an anti-HCV core monoclonal antibody) in amino acid sequence by the addition (e.g., insertion), deletion, or conservative substitution of amino acids, but that retains the biological activity of the given protein. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity and degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be

identified, in part, by considering the hydropathic index of amino acids, as understood in the art (see, e.g., Kyte et al, J. Mol. Biol. 157: 105-132 (1982)). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids also can be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity (see, e.g., U.S. Patent No. 4,554,101, which is incorporated herein by reference). Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. In one aspect, substitutions are performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to describe a polypeptide or fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, e.g., the ability to bind to IL-18. Use of "variant" herein is intended to encompass fragments of a variant unless otherwise contradicted by context.

The antibodies or antigen binding fragments may also be produced by genetic engineering. For example, the technology for expression of both heavy and light chain genes in E. coli is the subject of the PCT patent applications; publication number WO 901443, WO901443, and WO 9014424 and in Huse et al, 1989 Science 246:1275 1281. The present disclosure also encompasses an isolated recombinant vector comprising a nucleic acid molecule as described herein, as well as a host cell comprising such a recombinant vector. A vector is a nucleic acid molecule, which may be a construct, capable of transporting another nucleic acid to which it has been linked. A vector may include any preferred or required operational elements. Preferred vectors are those for which the restriction sites have been described and which contain the operational elements needed for transcription of the nucleic

acid sequence. Such operational elements include for example at least one suitable promoter, at least one operator, at least one leader sequence, at least one terminator codon, and any other DNA sequences necessary or preferred for appropriate transcription and subsequent translation of the nucleic acid sequence. Such vectors contain at least one origin of replication recognized by the host organism along with at least one selectable marker and at least one promoter sequence capable of initiating transcription of the nucleic acid sequence. A vector may be a plasmid into which additional DNA segments may be ligated. A vector may be a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as a plasmid is the most commonly used form of vector. However, the present disclosure is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

Sequences that are operably linked are in a relationship permitting them to function in their intended manner. A control sequence operably linked to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Operably linked sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. Expression control sequences are polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the

host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. Control sequences include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

A host cell may be transformed with a vector that introduces exogenous DNA into a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Transformed cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, and cells which transiently express the inserted DNA or RNA for limited periods of time.

Suitable host organisms include for example a eukaryotic cell system such as but not limited to cell lines such as HeLa, MRC-5 or CV-1. Host organisms such as host cells are cultured under conditions appropriate for amplification of the vector and expression of the protein, as well known in the art. Expressed recombinant proteins may be detected by any of a number of methods also well known in the art.

"Humanized" chimeric antibodies are described herein below. General reviews of "humanized" chimeric antibodies are provided by Morrison S., 1985 Science 229:1202 and by Oi et al, 1986 BioTechniques 4:214. Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones et al, 1986 Nature 321:552; Verhoeyan et al., 1988 Science 239:1534; Biedleret al. 1988 J. Immunol. 141:4053, the entire disclosures of which are incorporated herein by reference).

C. Engineered and Derivatized Binding Proteins

The present disclosure also pertains to engineered and derivatized binding proteins including dual variable domain immunoglobulin (DVD-Ig) binding proteins comprising one or more anti-HCV monoclonal antibodies as described herein.

1. DVD Binding Proteins

The monoclonal antibodies described herein may be used for example to construct DVD-Ig' s with unique binding affinities for HCV core protein, as described for example in

U.S. Patent No. 7,612,181, the entire disclosure of which is hereby incorporated by reference. DVD-Ig binding proteins are capable of binding one or more targets. Preferably the binding protein comprises a polypeptide chain comprising VDl-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, XI represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. The binding protein can be generated using various techniques.

A binding protein can comprise multiple polypeptide chains. A binding can comprise for example four polypeptide chains, of which two comprise VDl-(Xl)n-VD2-C-(X2)n, with VD1 a first heavy chain variable domain, VD2 a second heavy chain variable domain, C a heavy chain constant domain, XI a linker with the proviso that it is not CHI, and X2 an Fc region; and two polypeptide chains comprise VDl-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, XI is a linker with the proviso that it is not CHI, and X2 does not comprise an Fc region; and n is 0 or 1. The four polypeptide chains of such a binding protein form four functional antigen binding sites. The binding protein is for example capable of binding HCV core protein. The binding protein can be capable of modulating a biological function of HCV core protein, or of neutralizing HCV core protein. Exemplary such binding proteins have at least one heavy chain variable domain an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9, and at least one light chain variable domain comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10. The binding protein can comprise for example at least one amino acid sequence selected from the group consisting of SEQ ID NOS: 48-79.

The variable domains of a DVD binding protein can be obtained from parent antibodies, including polyclonal and monoclonal antibodies capable of binding antigens of interest. The monoclonal antibodies that specifically bind to HCV core protein described herein are suitable parent antibodies. Generally, antibodies used for the DVD binding protein may be naturally occurring or may be generated by recombinant technology.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those as described herein for preparing the anti-HCV core protein monoclonal antibodies, and those known in the art and taught, for example, in Harlow et al.,

Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed in Example 1 below. Hybridomas may be cultured and expanded in vivo in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture in vitro. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art. In a preferred embodiment, the hybridomas are mouse hybridomas. In another preferred embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an antibody capable of binding a specific antigen.

Recombinant monoclonal antibodies are also generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, PCT Publication WO 92/0255 1 and Babcock, J. S. et al. (1996) Proc. Natl. Acad. Sci. USA 93:7843-7848. In this method, single cells secreting antibodies of interest, e.g., lymphocytes derived from an immunized animal, are identified, and, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (e.g., human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from in vivo selected lymphocytes, can then undergo further analysis and selection in vitro, for example by panning the transfected cells to isolate cells expressing antibodies to the antigen of interest. The amplified immunoglobulin sequences further can be manipulated in vitro, such as by in vitro affinity maturation methods such as those described in PCT Publication WO 97/29131 and PCT Publication WO 00/56772.

Monoclonal antibodies are also produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an antigen of interest. In a preferred embodiment, the non-human animal is a XENOMOUSE® transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al. Nature Genetics 7:13-21 (1994) and U.S. Patent Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, published Jul. 25, 1991, WO 94/02602, published Feb. 3, 1994, WO 96/34096 and WO 96/33735, both published Oct. 31, 1996, WO 98/16654, published Apr. 23, 1998, WO 98/24893, published Jun. 11, 1998, WO 98/50433, published Nov. 12, 1998, WO 99/45031, published Sep. 10, 1999, WO 99/53049, published Oct. 21, 1999, WO 00 09560, published Feb. 24, 2000 and WO 00/037504, published Jun. 29, 2000. The XENOMOUSE® transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human Mabs. The XENOMOUSE® transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al, Nature Genetics 15:146-156 (1997), Green and Jakobovits J. Exp. Med. 188:483-495 (1998), the disclosures of which are hereby incorporated by reference.

In vitro methods also can be used to make the parent antibodies, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT Publication No. WO 92/18619; Dower et al. PCT Publication No. WO 91/17271; Winter et al. PCT Publication No. WO 92/20791; Markland et al. PCT Publication No. WO 92/15679; Breitling et al. PCT Publication No. WO 93/01288; McCafferty et al. PCT Publication No. WO 92/01047; Garrard et al. PCT Publication No. WO 92/09690; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum Antibod Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; McCafferty et al, Nature (1990) 348:552-554; Griffiths et al. (1993) EMBO J. 12:725-734; Hawkins et al. (1992) J Mol Biol 226:889-896; Clackson et al. (1991) Nature 352:624-628; Gram et al. (1992) PNAS 89:3576-3580; Garrad et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) Nuc Acid Res 19:4133-4137; and Barbas et al. (1991) PNAS 88:7978-7982, US patent application publication 20030186374,

and PCT Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

Parent antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies as described herein include those disclosed in Brinkman et al, J. Immunol. Methods 182:41-50 (1995); Ames et al, J. Immunol. Methods 184:177-186 (1995); Kettleborough et al, Eur. J. Immunol. 24:952-958 (1994); Persic et al, Gene 187 9-18 (1997); Burton et al, Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al, BioTechniques 12(6):864-869 (1992); and Sawai et al, AJRI 34:26-34 (1995); and Better et al, Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston et al, Methods in Enzymology 203:46-88

(1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al, Science 240:1038-1040 (1988).

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of parent antibodies. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 by Szostak and Roberts, and in Roberts, R. W. and Szostak, J. W. (1997) Proc. Natl. Acad. Sci. USA 94:12297-12302. In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by in vitro translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (e.g., a combinatorial library) based on the properties of the encoded peptide or protein, e.g., antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (e.g., in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation in vitro of recombinant antibodies, as described above.

In another approach the parent antibodies can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the parent antibodies include those disclosed in Wittrup, et al. U.S. Patent No. 6,699,658 incorporated herein by reference.

The antibodies described above can be further modified to generate CDR grafted and Humanized parent antibodies. CDR-grafted parent antibodies comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of murine antibodies capable of binding antigen of interest. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody

is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, it is preferable that the human variable framework that is chosen to replace the murine variable framework apart from the CDRs has at least a 65% sequence identity with the murine antibody variable region framework. It is more preferable that the human and murine variable regions apart from the CDRs have at least 70% sequence identify. It is even more preferable that the human and murine variable regions apart from the CDRs have at least 75% sequence identity. It is most preferable that the human and murine variable regions apart from the CDRs have at least 80% sequence identity. Methods for producing such antibodies are known in the art (see EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al, Protein Engineering 7(6):805-814 (1994); Roguska et al, PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,352).

Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez-/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/.about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH-05/kuby05.html; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uky.about.mrc7/m-ikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLiriks/Immunology.html.www.immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.-html; www.biotech.ufl. edu/.about.hcl/; www.pebio.com/pa/340913/340913.html-; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.acjp/.about.yasuhito-/Eli sa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/lin-ks.html; www.biotech.ufl.edu/. about. fee1/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP-Start.html; baserv.uci.kun.nl/.aboutjraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/pu-blic/INTRO.html; www.ibt.unam.mx/virV_-mice.html;

imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/.about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/.about.honegger/AHOseminar/SlideOl.html; www.cryst.bbk.ac.uk/.about.ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/.about.mrc7/humanisation/T AHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc. cam.ac.uk/.abo-ut.fmolina/Webpages/Pept/spottech.html; www.jerini.de/fr roducts.htm; www.patents.ibm.con/ibm.html.Kabat et al, Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al, U.S. Patent No. 5,585,089; Riechmann et al, Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al, Nature 321:522 (1986); Verhoeyen et al, Science 239:1534 (1988)), Sims et al, J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al, Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al, J. Immunol. 151:2623 (1993), Padlan, Molecular

Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al, PNAS 91:969-973 (1994); PCT publication WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB9 1/01 134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Patent Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567, each entirely incorporated herein by reference, included references cited therein.

Parent monoclonal antibodies may be selected from various monoclonal antibodies capable of binding specific targets including, or in addition to, HCV proteins, as well known in the art. These include, but are not limited to anti-TNF antibody (U.S. Patent No. 6,258,562), anti-IL-12 and or anti-IL-12p40 antibody (U.S. Patent No. 6,914,128); anti-IL-18 antibody (US 2005/0147610 Al), anti-C5, anti-CBL, anti-CD147, anti-gpl20, anti-VLA4, anti-CD 11a, anti-CD 18, anti-VEGF, anti-CD40L, anti-Id, anti-ICAM- 1, anti-CXCL13, anti-CD2, anti-EGFR, anti-TGF-beta 2, anti-E-selectin, anti-Fact VII, anti-Her2/neu, anti-F gp, anti-CDl l/18, anti-CD14, anti-ICAM-3, anti-CD80, anti-CD4, anti-CD3, anti-CD23, anti-beta2-integrin, anti-alpha4beta7, anti-CD52, anti-HLA DR, anti-CD22, anti-CD20, anti-MIF, anti-CD64 (FcR), anti-TCR alpha beta, anti-CD2, anti-Hep B, anti-CA 125, anti-EpCAM, anti-gpl20, anti-CMV, anti-gpllbllla, anti-IgE, anti-CD25, anti-CD33, anti-HLA, anti-VNRintegrin, anti-IL-1 alpha, anti-IL-lbeta, anti-IL-1 receptor, anti-IL-2 receptor, anti-IL-4, anti-IL4 receptor, anti-IL5, anti-IL-5 receptor, anti-IL-6, anti-IL-8, anti-IL-9, anti-IL-13, anti-IL-1 3 receptor, anti-IL-1 7, and anti-IL-23 (see Presta LG. 2005 Selection, design, and engineering of therapeutic antibodies J Allergy Clin Immunol. 116:73 1-6 and Clark, M., "Antibodies for Therapeutic Applications," Department of Pathology, Cambridge University, UK, 15 Oct. 2000, published online at M. Clark's home page at the website for the Department of Pathology, Cambridge University).

Parent monoclonal antibodies may also be selected from various therapeutic antibodies approved for use, in clinical trials, or in development for clinical use, particularly those that may be applicable in treating symptoms of HCV infection, or in treating conditions or diseases that co-exist with HCV infection, such as cancer, including particularly hepatocellular carcinoma. Such therapeutic antibodies include, but are not limited to, RITUXAN®, IDEC/Genentech/Roche) (see for example U.S. Patent No. 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HUMAX-

CD20®, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in U.S. Patent No. 5,500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), HumaLYM (Intracel), and PRO70769 (PCT/US2003/040426, entitled "Immunoglobulin Variants and Uses Thereof), trastuzumab (HERCEPTIN®, Genentech) (see for example U.S. Patent No. 5,677, 17 1), a humanized anti-Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, OMNITARG®), currently being developed by Genentech; an anti-Her2 antibody described in U.S. Patent No. 4,753,894; cetuximab (ERBITUX®, Imclone) (U.S. Patent No. 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (U.S. Patent No. 6,235,883), currently being developed by Abgenix-Immunex-Amgen; HUMAX-EGFR.TM. (U.S. Ser. No. 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (U.S. Patent No. 5,558,864; Murthy et al. 1987, Arch Biochem Biophys. 252(2):549-60; Rodeck et al, 1987, J Cell Biochem. 35(4):3 15-20; Kettleborough et al, 1991, Protein Eng. 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al, 1993, J. Cell Biophys. 1993, 22(1-3): 129-46; Modjtahedi et al, 1993, Br J Cancer. 1993, 67(2):247-53; Modjtahedi et al, 1996, Br J Cancer, 73(2):228-35; Modjtahedi et al, 2003, Int J Cancer, 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (U.S. Patent No. 5,891,996; U.S. Patent No. 6,506,883; Mateo et al, 1997, Immunotechnology, 3(1):71-81); mAb-806 (Ludwig Institute for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, Proc Natl Acad Sci USA. 100(2): 639-44); KSB-102 (KS Biomedix); MRl-1 (IVAX, National Cancer Institute) (PCT WO 016293 1A2); and SCIOO (Scancell) (PCT WO 01/88138); alemtuzumab (CAMPATH®, Millennium), a humanized monoclonal antibody currently approved for treatment of B-cell chronic lymphocytic leukemia; muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/ Johns on & Johnson, ibritumomab tiuxetan (ZEVALIN®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (MYLOTARG®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (AMEVIVE®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (REOPRO®), developed by Centocor/Lilly, basiliximab (SIMULECT®), developed by Novartis, palivizumab (SYNAGIS®), developed by Medimmune, infliximab (REMICADE®), an anti-TNF alpha antibody developed by Centocor, adalimumab (HUMIRA®), an anti-TNF alpha antibody developed by Abbott, HUMICADE®, an anti-TNFalpha antibody developed by Celltech, etanercept (ENBREL®),

an anti-TNF alpha Fc fusion developed by Immunex/Amgen, ABX-CBL, an anti-CD 147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, 90Y-muHMFGl), an anti-MUCl in development by Antisoma, Therex (R1550), an anti-MUCl antibody being developed by Antisoma, AngioMab (AS 1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS 1407) being developed by Antisoma, ANTEGREN® (natalizumab), an anti-alpha-4-beta- 1 (VLA4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF-.beta.2 antibody being developed by Cambridge Antibody Technology, J695, an anti-IL-12 antibody being developed by Cambridge Antibody Technology and Abbott, CAT- 192, an anti-TGF.beta.l antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxinl antibody being developed by Cambridge Antibody Technology, LYMPHOSTAT-B® an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-RlmAb, an anti-TRAIL-Rl antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc., AVASTIN® bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, XOLAIR® (Omalizumab), an anti-IgE antibody being developed by Genentech, RAPTIVA® (Efalizumab), an anti-CD 11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millennium Pharmaceuticals, HUMAX CD4®, an anti-CD4 antibody being developed by Genmab, HUMAX.TM.-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HUMAX.TM.-Inflam, being developed by Genmab and Medarex, HUMAX.TM.-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GlycoSciences, HUMAX.TM.-Lymphoma, being developed by Genmab and Amgen, HUMAX.TM.-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti-CD4 antibody being developed by IDEC Pharmaceuticals, IDEC- 114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC- 152, an anti-CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being

developed by Imclone, IMC-1C1 1, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-CIDE® (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LYMPHOCIDE® (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, OSIDEM® (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HUMAX®-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNFa antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MORI 0 1 and MORI 02, anti-intercellular adhesion molecule- 1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, NUVION® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HUZAF®, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti-a 5.beta.l Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody being developed by Xoma, XOLAIR® (Omalizumab) a humanized anti-IgE antibody developed by Genentech and Novartis, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma, all of the above-cited references in this paragraph are expressly incorporated herein by reference.

2. Construction of DVD Molecules

The dual variable domain immunoglobulin (DVD-Ig) molecule is designed such that two different light chain variable domains (VL) from the two different parent mAbs are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CHI and Fc region (FIG.l). The variable domains can be obtained using recombinant DNA techniques from a parent antibody generated by any one of the methods described above. In a preferred embodiment the variable domain is a murine heavy or light chain variable domain. More

preferably the variable domain is a CDR grafted or a humanized variable heavy or light chain domain. Most preferably the variable domain is a human heavy or light chain variable domain.

The first and second variable domains may be linked directly to each other using recombinant DNA techniques. Alternatively, the variable domains may be linked via a linker sequence. Preferably two variable domains are linked. Three or more variable domains may also be linked directly or via a linker sequence. The variable domains may bind the same antigen or may bind different antigens. DVD binding proteins may include one immunoglobulin variable domain and one non-immunoglobulin variable domain such as a ligand binding domain of a receptor, and active domain of an enzyme. DVD molecules may also comprise two or more non-Ig domains.

The linker sequence may be a single amino acid or a polypeptide sequence. Preferably the linker sequence is selected from the group consisting of SEQ ID NO: 25 through SEQ ID NO: 50 as provided herein above. The choice of linker sequences is based on crystal structure analysis of several Fab molecules. There is a natural flexible linkage between the variable domain and the CH l/CL constant domain in Fab or antibody molecular structure. This natural linkage comprises approximately 10-12 amino acid residues, contributed by 4-6 residues from C-terminus of V domain and 4-6 residues from the N-terminus of CL/CH1 domain. DVD Igs as described herein were generated using N-terminal 5-6 amino acid residues, or 11-12 amino acid residues, of CL or CHI as linker in light chain and heavy chain of DVD-Ig, respectively. The N-terminal residues of CL or CHI domains, particularly the first 5-6 amino acid residues, adopt a loop conformation without strong secondary structures, therefore can act as flexible linkers between the two variable domains. The N-terminal residues of CL or CH I domains are natural extension of the variable domains, as they are part of the Ig sequences, therefore minimize to a large extent any immunogenicity potentially arising from the linkers and junctions.

Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains; the light chain linkers can be from CK or Cλ; and the heavy chain linkers can be derived from CHI of any isotypes, including Cγ1, Cy2, Cy3, Cy4, Cal , Ca2, Cδ, Ce, and Cv. Linker sequences may also be derived from other proteins such as Ig-like proteins, (e.g. TCR, FcR, KIR); G/S based sequences (e.g G4S **repeats** (SEP ID NO: 47)'): hinge region-derived sequences; and other natural sequences from other proteins.

An exemplary DVD binding protein includes a constant domain linked to the two linked variable domains using recombinant DNA techniques. A sequence comprising linked heavy chain variable domains is for example linked to a heavy chain constant domain and a sequence comprising linked light chain variable domains is linked to a light chain constant domain. Preferably the constant domains are human heavy chain constant domain and human light chain constant domain respectively. Most preferably the DVD heavy chain is further linked to an Fc region. The Fc region may be a native sequence Fc region, or a variant Fc region. Most preferably the Fc region is a human Fc region. In a preferred embodiment the Fc region includes Fc region from IgGl, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

An exemplary DVD binding protein comprises two heavy chain DVD polypeptides and two light chain DVD polypeptides combined to form a DVD-Ig molecule. Detailed description of a variety of specific DVD-Ig molecules capable of binding specific targets, and methods of making the same, is provided in U.S. Patent No. 7,612,181.

3. Production of DVD Proteins

DVD binding proteins may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the DVD heavy and DVD light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the DVD proteins as described herein in either prokaryotic or eukaryotic host cells, expression of DVD proteins in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active DVD protein.

Preferred mammalian host cells for expressing the recombinant antibodies as described herein include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding DVD proteins are introduced into mammalian host cells, the DVD proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the DVD proteins in the host cells or, more preferably, secretion of the

DVD proteins into the culture medium in which the host cells are grown. DVD proteins can be recovered from the culture medium using standard protein purification methods.

In a preferred system for recombinant expression of DVD binding proteins, a recombinant expression vector encoding both the DVD heavy chain and the DVD light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the DVD heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the DVD heavy and light chains and intact DVD protein is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the DVD protein from the culture medium. Still further the disclosure provides a method of synthesizing a DVD protein as described herein by culturing a host cell as described herein in a suitable culture medium until a DVD protein as described herein is synthesized. The method can further comprise isolating the DVD protein from the culture medium.

An important feature of a DVD-Ig is that it can be produced and purified in a similar way as a conventional antibody. The production of DVD-Ig results in a homogeneous, single major product with desired dual-specific activity, without any sequence modification of the constant region or chemical modifications of any kind. Other previously described methods to generate "bi-specific", "multi-specific", and "multi-specific multivalent" full length binding proteins do not lead to a single primary product but instead lead to the intracellular or secreted production of a mixture of assembled inactive, mono-specific, multi-specific, multivalent, full length binding proteins, and multivalent full length binding proteins with combination of different binding sites. As an example, based on the design described by Miller and Presta (PCT publication WO2001/077342(Al), there are 16 possible combinations of heavy and light chains. Consequently only 6.25% of protein is likely to be in the desired active form. Separation of fully active forms of the protein from inactive and partially active forms of the protein using standard chromatography techniques, typically used in large scale manufacturing, is yet to be demonstrated.

Surprisingly the design of the "dual-specific multivalent full length binding proteins" as described herein leads to a dual variable domain light chain and a dual variable domain heavy chain, which assemble primarily to the desired "dual-specific multivalent full length binding proteins".

At least 50%, preferably 75% and more preferably 90% of the assembled, and expressed dual variable domain immunoglobulin molecules are the desired dual-specific tetravalent protein. This aspect particularly enhances the commercial utility of DVD binding proteins such as those described herein. A method to express a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single primary product of a "dual-specific tetravalent full length binding protein" is provided in U.S. Patent No. 7,612,181.

4. Derivatized Binding Proteins

Binding proteins as described herein can be labeled. A labeled binding protein comprises a binding protein, which may be a monoclonal antibody or a DVD binding protein as described herein, which is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled DVD binding protein can be derived by functionally linking a DVD binding protein (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the binding protein with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which a binding protein may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-l-napthalenesulfonyl chloride, phycoerythrin and the like. A binding protein may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When a binding protein is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. A DVD binding protein may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Binding proteins as described herein, including any monoclonals and DVD binding proteins, may be crystallized, and used in various formulations and compositions comprising such crystals. A crystallized binding protein may have a greater half-life in vivo than the soluble counterpart of the binding protein. The binding protein may retain biological activity after crystallization. Crystallized binding protein as described herein may be produced by methods known in the art and as disclosed for example in WO 02072636, the entire disclosure of which is incorporated herein by reference.

A glycosylated binding protein may be prepared wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent in vivo protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, Biotechnol. Prog. 2 1 (2005), pp. 11-16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M. S., et al., Mol. Immunol. (1993) 30:1361-1367), or result in increased affinity for the antigen (Wallick, S. C , et al, Exp. Med. (1988) 168: 1099-1 109; Wright, A., et al, EMBO J. (1991) 10:2717 2723). Glycosylation site mutants can be generated in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity but have increased or decreased binding activity are another object as described herein.

Glycosylation of the antibody or antigen-binding portion of a binding protein can be modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation

may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Patent Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, a modified binding protein can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies as described herein to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) J. Biol. Chem. 277:26733-26740; Umana et al. (1999) Nat. Biotech. 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80, the disclosure of each of which is incorporated herein by reference in its entirety.

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful according to the present disclosure may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life in vivo after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion,

recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S patent applications 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

In addition to the binding proteins, an anti-idiotypic (anti-Id) antibody can be prepared, which is specific for the binding proteins described herein. An anti-Id antibody is an antibody that recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal with the binding protein or a CDR containing region thereof. The immunized animal will recognize, and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. Preferably, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

5. Uses of DVD-Ig Binding Proteins

Given their ability to bind to two or more antigen binding sites, including two or more antigens, the DVD binding proteins can be used to detect one or more antigens (e.g., in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The DVD-Ig binding protein is directly or indirectly labeled with a

detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{113}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm.

DVD binding proteins preferably are capable of neutralizing the activity of the antigens both in vitro and in vivo. Accordingly, such DVD-Igs can be used to inhibit antigen activity, e.g., in a cell culture containing the antigens, in human subjects or in other mammalian subjects having the antigens with which a binding protein as described herein cross-reacts. The DVD binding proteins can be used in a method for reducing antigen activity in a subject suffering from a disease or disorder in which the antigen activity, e.g. HCV core protein activity, is detrimental. A DVD binding protein incorporating one or more anti-HCV core protein monoclonal antibodies can be administered to a human subject for therapeutic purposes.

As used herein, the term "a disorder in which antigen activity is detrimental" is intended to include diseases and other disorders in which the presence of the antigen in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which antigen activity is detrimental is a disorder in which reduction of antigen activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of the antigen in a biological fluid of a subject suffering from the disorder (e.g., an increase in the concentration of antigen in serum, plasma, synovial fluid, etc. of the subject). Non-limiting examples of disorders that can be treated with the binding proteins as described herein include those disorders discussed below and in the section pertaining to pharmaceutical and diagnostic compositions of the binding proteins including the monoclonal antibodies as described herein.

The DVD-Igs may bind HCV core protein alone or multiple antigens including HCV core protein and one or more other antigens. Other such antigens include, but are not limited to, the targets listed in the following databases, which databases are incorporated herein by reference. These target databases include those listings: Therapeutic targets (http://xin.cz3.nus.edu.sg/group/cjttd/ttd.asp); Cytokines and cytokine receptors (http://www.cytokinewebfacts.com/, http://www.copewithcytokines.de/cope.cgi, and http://cmbi.bjmu.edu.cn/cmbidata/cgf/CGF_Database/cytokine.medic.kumamoto-u.ac.jp/CFC/indexR.html); Chemokines (http://cytokine.medic.kumamoto-u.ac jp/CFC/CK/Chemokine.html); Chemokine receptors and GPCRs (http://csp.medic.kumamoto-u.acjp/CSP/Receptor.html, http://www.gpcr.org/7tm/); Olfactory Receptors (http://senselab.med.yale.edu/senselab/ORDB/default.asp); Receptors (http://www.iuphar-db.org/iuphar-rd/list/index.htm); Cancer targets (http://cged.hgcjp/cgi-bin/inputcgi); Secreted proteins as potential antibody targets (http://spd.cbi.pku.edu.cn/); Protein kinases (http://spd.cbi.pku.edu.cn/), and Human CD markers (http://content.labvelocity.eom/tools/6/1226/CD_table_final_locked.pdf) and (Zola H, 2005 CD molecules 2005: human cell differentiation molecules Blood, 106:3 123-6). For example, DVD-Igs are useful as therapeutic agents to simultaneously block two different targets to enhance efficacy/safety and/or increase patient coverage. Such targets may include soluble targets (IL-13 and TNF) and cell surface receptor targets (VEGFR and EGFR). It can also be used to induce redirected cytotoxicity between tumor cells and T cells (Her2 and CD3) for cancer therapy, or between autoreactive cell and effector cells for autoimmune/transplantation, or between any target cell and effector cell to eliminate disease-causing cells in any given disease.

Additionally, DVD-Igs can be employed for tissue-specific delivery (target a tissue marker and a disease mediator for enhanced local PK thus higher efficacy and/or lower toxicity), including intracellular delivery (targeting an internalizing receptor and a intracellular molecule), delivering to inside brain (targeting transferrin receptor and a CNS disease mediator for crossing the blood-brain barrier). DVD-Ig can also serve as a carrier protein to deliver an antigen to a specific location via binding to a non-neutralizing epitope of that antigen and also to increase the half-life of the antigen. Furthermore, DVD-Ig can be designed to either be physically linked to medical devices implanted into patients or target these medical devices (see Burke, Sandra E.; Kuntz, Richard E.; Schwartz, Lewis B., Zotarolimus (ABT-578) eluting stents. Advanced Drug Delivery Reviews (2006), 58(3), 437-

446; Surface coatings for biological activation and functionalization of medical devices, Hildebrand, H. F.; Blanchemain, N.; Mayer, G.; Chai, F.; Lefebvre, M.; Boschin, F., Surface and Coatings Technology (2006), 200(22-23), 6318-6324; Drug/device combinations for local drug therapies and infection prophylaxis, Wu, Peng; Grainger, David W., Biomaterials (2006), 27(1 1), 2450-2467; Mediation of the cytokine network in the implantation of orthopedic devices., Marques, A. P.; Hunt, J. A.; Reis, Rui L., Biodegradable Systems in Tissue Engineering and Regenerative Medicine (2005), 377-397). Briefly, directing appropriate types of cell to the site of medical implant may promote healing and restoring normal tissue function. Alternatively, inhibition of mediators (including but not limited to cytokines), released upon device implantation by a DVD coupled to or target to a device is also provided. For example, Stents have been used for years in interventional cardiology to clear blocked arteries and to improve the flow of blood to the heart muscle. However, traditional bare metal stents have been known to cause restenosis (re-narrowing of the artery in a treated area) in some patients and can lead to blood clots. Recently, an anti-CD34 antibody coated stent has been described which reduced restenosis and prevents blood clots from occurring by capturing endothelial progenitor cells (EPC) circulating throughout the blood. Endothelial cells are cells that line blood vessels, allowing blood to flow smoothly. The EPCs adhere to the hard surface of the stent forming a smooth layer that not only promotes healing but prevents restenosis and blood clots, complications previously associated with the use of stents (Aoji et al. 2005 J Am Coll Cardiol. 45(10): 1574-9). In addition to improving outcomes for patients requiring stents, there are also implications for patients requiring cardiovascular bypass surgery. For example, a prosthetic vascular conduit (artificial artery) coated with anti-EPC antibodies would eliminate the need to use arteries from patients' legs or arms for bypass surgery grafts. This would reduce surgery and anesthesia times, which in turn will reduce coronary surgery deaths. DVD-Ig are designed in such a way that it binds to a cell surface marker (such as CD34) as well as a protein (or an epitope of any kind, including but not limited to lipids and polysaccharides) that has been coated on the implanted device to facilitate the cell recruitment. Such approaches can also be applied to other medical implants in general. Alternatively, DVD-Igs can be coated on medical devices and upon implantation and releasing all DVDs from the device (or any other need which may require additional fresh DVD-Ig, including aging and denaturation of the already loaded DVD-Ig) the device could be reloaded by systemic administration of fresh DVD-Ig to the patient, where the DVD-Ig is designed to binds to a target of interest (a cytokine, a cell

surface marker (such as CD34) etc.) with one set of binding sites and to a target coated on the device (including a protein, an epitope of any kind, including but not limited to lipids, polysaccharides and polymers) with the other. This technology has the advantage of extending the usefulness of coated implants.

D. Pharmaceutical and Diagnostic Compositions

The present disclosure provides pharmaceutical and diagnostic compositions comprising one or more anti-HCV core binding proteins disclosed herein as an active ingredient. Pharmaceutical or diagnostic compositions may comprise any monoclonal antibody or DVD-Ig binding protein described herein, or any combination thereof, and a pharmaceutically acceptable carrier, diluent and/or excipient. Generally, the pharmaceutical and diagnostic compositions are prepared by combining the active ingredient with the carrier, diluent and/or excipient.

The pharmaceutical compositions comprising binding proteins as described herein are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more binding proteins as described herein. In another embodiment, the pharmaceutical composition comprises one or more binding proteins as described herein and one or more prophylactic or therapeutic agents other than binding proteins as described herein for treating a disorder. Preferably, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The binding proteins can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a binding protein as described herein and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise

minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Various delivery systems are known and can be used to administer one or more binding proteins or the combination of one or more binding proteins as described herein, and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent as described herein include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, a binding protein as described herein, combination therapy, or a composition as described herein is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). Prophylactic or therapeutic agents as described herein can be administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

The prophylactic or therapeutic agents as described herein can be administered locally to the area in need of treatment; which may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as silastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. An effective amount of one or more antibodies as described herein antagonists can be administered locally

to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. An effective amount of one or more binding proteins as described herein can be administered locally to the affected area of a subject, in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than a binding protein as described herein, to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

The prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. For example, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al, 1980, Surgery 88:507; Saudek et al, 1989, N. Engl. J. Med. 321:574). Polymeric materials can be used to achieve controlled or sustained release of the therapies using any of the binding proteins as described herein (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al, 1985, Science 228:190; During et al, 1989, Ann. Neurol. 25:351; Howard et al, 1989, J. Neurosurg. 7 1:105); U.S. PatentNo. 5,679,377; U.S. PatentNo. 5,916,597; U.S. PatentNo. 5,912,015; U.S. PatentNo. 5,989,463; U.S. PatentNo. 5,128,326; PCT Publication No. WO 99/15 154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. An exemplary formulation uses a polymer in a sustained release formulation that is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Controlled release systems are discussed in a review by Langer (1990, Science 249: 1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents as described herein. See, e.g., U.S. PatentNo. 4,526,938, PCT publication WO 91/05548, PCT publication WO

96/20698, Ning et al., 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song et al, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397, Cleek et al, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in their entireties.

When a composition includes a nucleic acid encoding a prophylactic or therapeutic agent as described herein, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al, 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition can be formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. A composition can be formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection.

If the compositions are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous

to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

If the method of treatment comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use as described herein can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insulator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. If the method comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate) lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats);

emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of treatment may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/3 1346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, a binding protein as described herein, combination therapy, and/or composition as described herein is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

The method of treatment may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen- free water) before use.

The methods of treatment may additionally comprise administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

The methods of treatment encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium,

calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, the composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions as described herein can be packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. One or more of the prophylactic or therapeutic agents, or pharmaceutical compositions as described herein can be supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions as described herein is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions as described herein should be stored at between 2°C and 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions as described herein should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions as described herein is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2° C and 8° C in its original container.

The binding proteins as described herein can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml binding protein. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the binding proteins as described herein prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (e.g., antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (see WO2004078140, and US2006 104968 incorporated herein by reference).

The compositions as described herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the

antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The binding proteins as described herein can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, a binding protein as described herein may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell

gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound as described herein by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein as described herein is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in whichIL-12 activity is detrimental. For example, a binding protein as described herein may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more antibodies as described herein may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, a binding protein is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. application Ser. No. 09/428,082 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

In a specific embodiment, nucleic acid sequences encoding a binding protein as described herein or another prophylactic or therapeutic agent as described herein are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment as described herein, the nucleic acids produce their encoded antibody or prophylactic or therapeutic agent as described herein that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used with respect to the binding proteins as described herein. For general reviews of the methods of gene therapy, see Goldspiel et al, 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217;

May, 1993, TIBTECH 11(5): 155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). A detailed description of various methods of gene therapy is provided in US20050042664 A1 which is incorporated herein by reference in its entirety.

The binding proteins as described herein are useful in treating various diseases wherein the targets that are recognized by the binding proteins are detrimental. Such diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia greata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinemia), dilated cardiomyopathy, female infertility, ovarian

failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjogren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjorgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Thl Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial

infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aordic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chromic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia,

infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine-Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non-hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, Pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular

heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue. (see Peritt et al. PCT publication No. WO2002097048A2, Leonard et al, PCT publication No. W09524918 Al, and Salfeld et al, PCT publication No. WO00/56772A1).

The binding proteins as described herein can be used to treat humans suffering from liver diseases, in particular those associated with HCV infection, including cirrhosis, fibrosis and hepatocellular carcinoma.

A binding protein as described herein also can be administered with one or more additional therapeutic agents useful in the treatment of various diseases.

A binding protein as described herein can be used alone or in combination to treat such diseases. It should be understood that the binding proteins can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody as described herein. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition e.g., an agent that affects the viscosity of the composition.

It should further be understood that the combinations that are encompassed by the present disclosure are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this disclosure, can be the antibodies as described herein and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Non-limiting examples of therapeutic agents for HCV with which binding proteins of the as described herein can be combined include the following: Interferon-alpha-2a, Interferon-alpha-2b, Interferon-alpha conl, Interferon-alpha-nl, Pegylated interferon-alpha-2a, Pegylated interferon-alpha-2b, ribavirin, Peginterferon alfa-2b+ribavirin, Ursodeoxycholic Acid, Glycyrrhizic Acid, Thymalfasin, Maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets: HCV polymerase, HCV protease, HCV helicase, HCV IRES (internal ribosome entry site).

The pharmaceutical compositions as described herein may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding protein as described herein. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms as described herein are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an binding protein as described herein is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the

compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

E. Immunoassays

The anti-HCV core binding proteins, including monoclonal antibodies and DVD binding proteins as described herein, can also be used in immunoassays for diagnosing or prognosing hepatitis C virus infection in a mammal. As used throughout the present disclosure, "mammal" includes humans and non-human primates. It will be understood that a target analyte in the immunoassays and related methods is HCV core protein, although two or more target analytes may include a second analyte that is not HCV core protein. Immunoassays according to the present disclosure include such techniques commonly recognized in the art, including for example radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, immunohistochemical assay, immunoprecipitation and the like. Standard techniques known in the art for ELISA are well-known and described for example in Methods in Immunodiagnosis, 2nd Edition, Rose and Bigazzi, eds., John Wiley and Sons, 1980 and Campbell et al, Methods of Immunology, W. A. Benjamin, Inc., 1964, both of which are incorporated herein by reference. Immunoassays may be a direct, indirect, competitive, or noncompetitive immunoassay as described in the art (Oellerich, M. 1984. J. Clin. Chem. Clin. BioChem 22:895 904). Biological samples appropriate for such detection assays include, but are not limited to blood, plasma, serum, liver, saliva, lymphocytes or other mononuclear cells.

An immunoassay for determining the presence or amount of human hepatitis C virus in a sample may comprise, for example, providing a binding reagent comprising any one of the humanized monoclonal antibodies or DVD-Ig binding proteins as described herein, combining the monoclonal antibody or DVD-Ig binding reagent with the sample for a time sufficient for the reagent to bind to any human hepatitis C virus that may be present in the sample, and determining the presence or amount of human hepatitis C virus present in the sample based on specific binding of the binding reagent to the human hepatitis C virus core protein. The disclosure also encompasses an immunoassay device for detecting the presence or absence of human HCV in a sample, wherein the device comprises any of the humanized monoclonal antibodies described herein immobilized on a solid support. The anti-HCV core monoclonals and any analogs thereof may be prepared in the form of a kit, alone, or in combinations with other reagents such as secondary antibodies, for use in immunoassays.

Methods of detecting the presence of an analyte such as HCV core can comprise contacting the sample with at least one anti-HCV binding protein, including a monoclonal antibody or DVD-Ig as described herein. Any suitable assay as is known in the art can be used in the method. Examples include, but are not limited to, immunoassay, such as sandwich immunoassay (e.g., monoclonal, polyclonal and/or DVD-Ig sandwich immunoassays or any variation thereof (e.g., monoclonal/DVD-Ig, DVD-Ig/polyclonal, etc.), including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, MN)), competitive inhibition immunoassay (e.g., forward and reverse), fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogeneous chemiluminescent assay, etc. In a SELDI-based immunoassay, a capture reagent that specifically binds an analyte of interest such as HCV core (or a fragment thereof) is attached to the surface of a mass spectrometry probe, such as a pre-activated protein chip array. The analyte (or a fragment thereof) is then specifically captured on the biochip, and the captured analyte (or a fragment thereof) is detected by mass spectrometry. Alternatively, the analyte (or a fragment thereof) can be eluted from the capture reagent and detected by traditional MALDI (matrix-assisted laser desorption/ionization) or by SELDI. A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), is an example of a preferred immunoassay.

Methods well-known in the art for collecting, handling and processing urine, blood, serum and plasma, and other body fluids, are used in the practice of the present disclosure, for instance, when a DVD-Ig as described herein is employed as an immunodiagnostic reagent and/or in an analyte immunoassay kit. The test sample can comprise further moieties in addition to the analyte of interest, such as antibodies, antigens, haptens, hormones, drugs, enzymes, receptors, proteins, peptides, polypeptides, oligonucleotides and/or polynucleotides. For example, the sample can be a whole blood sample obtained from a subject. It can be necessary or desired that a test sample, particularly whole blood, be treated prior to immunoassay as described herein, e.g., with a pretreatment reagent. Even in cases where pretreatment is not necessary (e.g., most urine samples), pretreatment optionally can be done (e.g., as part of a regimen on a commercial platform).

The pretreatment reagent can be any reagent appropriate for use with the immunoassay and kits of the present disclosure. The pretreatment optionally comprises: (a) one or more solvents (e.g., methanol and ethylene glycol) and optionally, salt, (b) one or more solvents and salt, and optionally, detergent, (c) detergent, or (d) detergent and salt. Pretreatment reagents are known in the art, and such pretreatment can be employed, e.g., as used for assays on Abbott TDx, AxSYM®, and ARCHITECT® analyzers (Abbott Laboratories, Abbott Park, IL), as described in the literature (see, e.g., Yatscoff et al, Abbott TDx Monoclonal Antibody Assay Evaluated for Measuring Cyclosporine in Whole Blood, Clin. Chem. 36: 1969-1973 (1990), and Wallemacq et al, Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and EMIT Cyclosporine Assays, Clin. Chem. 45: 432-435 (1999)), and/or as commercially available. Additionally, pretreatment can be done as described in Abbott Laboratories' U.S. Patent No. 5,135,875, European Patent Pub. No. 0 471 293, U.S Provisional Patent App. 60/878,017, filed December 29, 2006, and U.S. Patent App. Pub. No. 2008/0020401 (incorporated by reference in its entirety for its teachings regarding pretreatment). The pretreatment reagent can be a heterogeneous agent or a homogeneous agent.

With use of a heterogeneous pretreatment reagent, the pretreatment reagent precipitates analyte binding protein (e.g., protein that can bind to an analyte or a fragment thereof) present in the sample. Such a pretreatment step comprises removing any analyte binding protein, e.g. anti-HCV core protein monoclonal antibody,vby separating from the precipitated analyte binding protein the supernatant of the mixture formed by addition of the pretreatment agent to sample. In such an assay, the supernatant of the mixture absent any binding protein is used in the assay, proceeding directly to the antibody capture step.

With use of a homogeneous pretreatment reagent there is no such separation step. The entire mixture of test sample and pretreatment reagent are contacted with a labeled specific binding partner for analyte (or a fragment thereof), such as a labeled anti-analyte antibody (or an antigenically reactive fragment thereof). The pretreatment reagent employed for such an assay typically is diluted in the pretreated test sample mixture, either before or during capture by the first specific binding partner. Despite such dilution, a certain amount of the pretreatment reagent is still present (or remains) in the test sample mixture during capture. According to the present disclosure, the labeled specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof).

In a heterogeneous format, after the test sample is obtained from a subject, a first mixture is prepared. The mixture contains the test sample being assessed for an analyte (or a fragment thereof) and a first specific binding partner, wherein the first specific binding partner and any analyte contained in the test sample form a first specific binding partner-analyte complex. Preferably, the first specific binding partner is an anti-analyte antibody or a fragment thereof. The first specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein. The order in which the test sample and the first specific binding partner are added to form the mixture is not critical. Preferably, the first specific binding partner is immobilized on a solid phase. The solid phase used in the immunoassay (for the first specific binding partner and, optionally, the second specific binding partner) can be any solid phase known in the art, such as, but not limited to, a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a film, a filter paper, a disc and a chip.

Also, the methods as described herein can be adapted for use in systems that utilize microparticle technology including in automated and semi-automated systems wherein the solid phase comprises a microparticle. Such systems include those described in pending U.S. patent application Ser. Nos. 425,651 and 425,643, which correspond to published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively, which are incorporated herein by reference.

After the mixture containing the first specific binding partner-analyte complex is formed, any unbound analyte is removed from the complex using any technique known in the art. For example, the unbound analyte can be removed by washing. Desirably, however, the first specific binding partner is present in excess of any analyte present in the test sample, such that all analyte that is present in the test sample is bound by the first specific binding partner.

After any unbound analyte is removed, a second specific binding partner is added to the mixture to form a first specific binding partner-analyte-second specific binding partner complex. The second specific binding partner is preferably an anti-analyte antibody that binds to an epitope on analyte that differs from the epitope on analyte bound by the first specific binding partner. Moreover, also preferably, the second specific binding partner is labeled with or contains a detectable label as described above. The second specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein.

Any suitable detectable label as is known in the art can be used. For example, the detectable label can be a radioactive label (such as ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, and ^{33}P), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachloro fluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, Introduction to Immunocytochemistry, 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, Handbook of Fluorescent Probes and Research Chemicals (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oregon. A fluorescent label can be used in FPIA (see, e.g., U.S. Patent Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803, which are hereby incorporated by reference in their entireties). An acridinium compound can be used as a detectable label in a homogeneous or heterogeneous chemiluminescent assay (see, e.g., Adamczyk et al, Bioorg. Med. Chem. Lett. 16: 1324-1328 (2006); Adamczyk et al, Bioorg. Med. Chem. Lett. 4: 2313-2317 (2004); Adamczyk et al, Biorg. Med. Chem. Lett. 14: 3917-3921 (2004); and Adamczyk et al, Org. Lett. 5: 3779-3782 (2003)).

A preferred acridinium compound is an acridinium-9-carboxamide. Methods for preparing acridinium 9-carboxamides are described in Mattingly, J. Biolumin. Chemilumin. 6: 107-114 (1991); Adamczyk et al, J. Org. Chem. 63: 5636-5639 (1998); Adamczyk et al, Tetrahedron 55: 10899-10914 (1999); Adamczyk et al, Org. Lett. 1: 779-781 (1999); Adamczyk et al, Bioconjugate Chem. 11: 714-724 (2000); Mattingly et al, In Luminescence Biotechnology: Instruments and Applications; Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77-105 (2002); Adamczyk et al, Org. Lett. 5: 3779-3782 (2003); and U.S. Patent Nos. 5,468,646, 5,543,524 and 5,783,699 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Another preferred acridinium compound is an acridinium-9-carboxylate aryl ester. An example of an acridinium-9-carboxylate aryl ester is 10-methyl-9-(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra et al, Photochem. Photobiol. 4: 1111-21 (1965); Razavi et al.,

Luminescence 15: 245-249 (2000); Razavi et al, Luminescence 15: 239-244 (2000); and U.S. Patent No. 5,241,070 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Further details regarding acridinium-9-carboxylate aryl ester and its use are set forth in US 2008-0248493.

Chemiluminescent assays (e.g., using acridinium as described above or other chemiluminescent agents) can be performed in accordance with the methods described in Adamczyk et al, Anal. Chim. Acta 579(1): 61-67 (2006). While any suitable assay format can be used, a microplate chemiluminometer (Mithras LB-940, Berthold Technologies U.S.A., LLC, Oak Ridge, TN) enables the assay of multiple samples of small volumes rapidly.

The order in which the test sample and the specific binding partner(s) are added to form the mixture for chemiluminescent assay is not critical. If the first specific binding partner is detectably labeled with a chemiluminescent agent such as an acridinium compound, detectably labeled first specific binding partner-analyte complexes form. Alternatively, if a second specific binding partner is used and the second specific binding partner is detectably labeled with a chemiluminescent agent such as an acridinium compound, detectably labeled first specific binding partner-analyte-second specific binding partner complexes form. Any unbound specific binding partner, whether labeled or unlabeled, can be removed from the mixture using any technique known in the art, such as washing.

Hydrogen peroxide can be generated in situ in the mixture or provided or supplied to the mixture (e.g., the source of the hydrogen peroxide being one or more buffers or other solutions that are known to contain hydrogen peroxide) before, simultaneously with, or after the addition of an above-described acridinium compound. Hydrogen peroxide can be generated in situ in a number of ways such as would be apparent to one skilled in the art.

Upon the simultaneous or subsequent addition of at least one basic solution to the sample, a detectable signal, namely, a chemiluminescent signal, indicative of the presence of analyte is generated. The basic solution contains at least one base and has a pH greater than or equal to 10, preferably, greater than or equal to 12. Examples of basic solutions include, but are not limited to, sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium hydroxide, calcium carbonate, and calcium bicarbonate. The amount of basic solution added to the sample depends on the concentration of the basic solution. Based on

the concentration of the basic solution used, one skilled in the art can easily determine the amount of basic solution to add to the sample.

The chemiluminescent signal that is generated can be detected using routine techniques known to those skilled in the art. Based on the intensity of the signal generated, the amount of analyte in the sample can be quantified. Specifically, the amount of analyte in the sample is proportional to the intensity of the signal generated. The amount of analyte present can be quantified by comparing the amount of light generated to a standard curve for analyte or by comparison to a reference standard. The standard curve can be generated using serial dilutions or solutions of known concentrations of analyte by mass spectroscopy, gravimetric methods, and other techniques known in the art. While the above is described with emphasis on use of an acridinium compound as the chemiluminescent agent, one of ordinary skill in the art can readily adapt this description for use of other chemiluminescent agents.

Analyte immunoassays generally can be conducted using any format known in the art, such as, but not limited to, a sandwich format. Specifically, in one immunoassay format, at least two antibodies are employed to separate and quantify analyte, such as human analyte, or a fragment thereof in a sample. More specifically, the at least two antibodies bind to different epitopes on an analyte (or a fragment thereof) forming an immune complex, which is referred to as a "sandwich." Generally, in the immunoassays one or more antibodies can be used to capture the analyte (or a fragment thereof) in the test sample (these antibodies are frequently referred to as a "capture" antibody or "capture" antibodies) and one or more antibodies can be used to bind a detectable (namely, quantifiable) label to the sandwich (these antibodies are frequently referred to as the "detection antibody," the "detection antibodies," the "conjugate," or the "conjugates"). Thus, in the context of a sandwich immunoassay format, a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein can be used as a capture antibody, a detection antibody, or both. For example, one DVD-Ig having a domain that can bind a first epitope on an analyte (or a fragment thereof) can be used as a capture antibody and/or another DVD-Ig having a domain that can bind a second epitope on an analyte (or a fragment thereof) can be used as a detection antibody. In this regard, a DVD-Ig having a first domain that can bind a first epitope on an analyte (or a fragment thereof) and a second domain that can bind a second epitope on an analyte (or a fragment thereof) can be used as a capture antibody and/or a detection antibody. Alternatively, one DVD-Ig having a first domain that can bind an epitope on a first analyte (or a fragment thereof) and a second

domain that can bind an epitope on a second analyte (or a fragment thereof) can be used as a capture antibody and/or a detection antibody to detect, and optionally quantify, two or more analytes. In the event that an analyte can be present in a sample in more than one form, such as a monomeric form and a dimeric/multimeric form, which can be homomeric or heteromeric, one DVD-Ig having a domain that can bind an epitope that is only exposed on the monomeric form and another DVD-Ig having a domain that can bind an epitope on a different part of a dimeric/multimeric form can be used as capture antibodies and/or detection antibodies, thereby enabling the detection, and optional quantification, of different forms of a given analyte. Furthermore, employing DVD-Igs with differential affinities within a single DVD-Ig and/or between DVD-Igs can provide an avidity advantage. In the context of immunoassays as described herein, it generally may be helpful or desired to incorporate one or more linkers within the structure of a DVD-Ig. When present, optimally the linker should be of sufficient length and structural flexibility to enable binding of an epitope by the inner domains as well as binding of another epitope by the outer domains. In this regard, if a DVD-Ig can bind two different analytes and one analyte is larger than the other, desirably the larger analyte is bound by the outer domains.

Generally speaking, a sample being tested for (for example, suspected of containing) analyte (or a fragment thereof) can be contacted with at least one capture antibody (or antibodies) and at least one detection antibody (which can be a second detection antibody or a third detection antibody or even a successively numbered antibody, e.g., as where the capture and/or detection antibody comprise multiple antibodies) either simultaneously or sequentially and in any order. For example, the test sample can be first contacted with at least one capture antibody and then (sequentially) with at least one detection antibody. Alternatively, the test sample can be first contacted with at least one detection antibody and then (sequentially) with at least one capture antibody. In yet another alternative, the test sample can be contacted simultaneously with a capture antibody and a detection antibody.

In the sandwich assay format, a sample suspected of containing analyte (or a fragment thereof) is first brought into contact with at least one first capture antibody under conditions that allow the formation of a first antibody/analyte complex. If more than one capture antibody is used, a first capture antibody/analyte complex comprising two or more capture antibodies is formed. In a sandwich assay, the antibodies, i.e., preferably, the at least one capture antibody, are used in molar excess amounts of the maximum amount of analyte (or a

fragment thereof) expected in the test sample. For example, from about 5 µg to about 1 mg of antibody per mL of buffer (e.g., microparticle coating buffer) can be used.

Competitive inhibition immunoassays, which are often used to measure small analytes because binding by only one antibody is required, comprise sequential and classic formats. In a sequential competitive inhibition immunoassay a capture antibody to an analyte of interest is coated onto a well of a microtiter plate or other solid support. When the sample containing the analyte of interest is added to the well, the analyte of interest binds to the capture antibody. After washing, a known amount of labeled (e.g., biotin or horseradish peroxidase (HRP)) analyte is added to the well. A substrate for an enzymatic label is necessary to generate a signal. An example of a suitable substrate for HRP is 3,3',5,5'-tetramethylbenzidine (TMB). After washing, the signal generated by the labeled analyte is measured and is inversely proportional to the amount of analyte in the sample. In a classic competitive inhibition immunoassay an antibody to an analyte of interest is coated onto a solid support (e.g., a well of a microtiter plate). However, unlike the sequential competitive inhibition immunoassay, the sample and the labeled analyte are added to the well at the same time. Any analyte in the sample competes with labeled analyte for binding to the capture antibody. After washing, the signal generated by the labeled analyte is measured and is inversely proportional to the amount of analyte in the sample.

Optionally, prior to contacting the test sample with the at least one capture antibody (for example, the first capture antibody), the at least one capture antibody can be bound to a solid support, which facilitates the separation of the first antibody/analyte (or a fragment thereof) complex from the test sample. The substrate to which the capture antibody is bound can be any suitable solid support or solid phase that facilitates separation of the capture antibody-analyte complex from the sample.

Examples include a well of a plate, such as a microtiter plate, a test tube, a porous gel (e.g., silica gel, agarose, dextran, or gelatin), a polymeric film (e.g., polyacrylamide), beads (e.g., polystyrene beads or magnetic beads), a strip of a filter/membrane (e.g., nitrocellulose or nylon), microparticles (e.g., latex particles, magnetizable microparticles (e.g., microparticles having ferric oxide or chromium oxide cores and homo- or hetero-polymeric coats and radii of about 1-10 microns). The substrate can comprise a suitable porous material with a suitable surface affinity to bind antigens and sufficient porosity to allow access by detection antibodies. A microporous material is generally preferred, although a gelatinous material in a hydrated state can be used. Such porous substrates are preferably in the form of

sheets having a thickness of about 0.01 to about 0.5 mm, preferably about 0.1 mm. While the pore size may vary quite a bit, preferably the pore size is from about 0.025 to about 15 microns, more preferably from about 0.15 to about 15 microns. The surface of such substrates can be activated by chemical processes that cause covalent linkage of an antibody to the substrate. Irreversible binding, generally by adsorption through hydrophobic forces, of the antigen or the antibody to the substrate results; alternatively, a chemical coupling agent or other means can be used to bind covalently the antibody to the substrate, provided that such binding does not interfere with the ability of the antibody to bind to analyte. Alternatively, the antibody can be bound with microparticles, which have been previously coated with streptavidin (e.g., DYNAL® Magnetic Beads, Invitrogen, Carlsbad, CA) or biotin (e.g., using Power-BindTM-SA-MP streptavidin-coated microparticles (Seradyn, Indianapolis, IN)) or anti-species-specific monoclonal antibodies. If necessary, the substrate can be derivatized to allow reactivity with various functional groups on the antibody. Such derivatization requires the use of certain coupling agents, examples of which include, but are not limited to, maleic anhydride, N-hydroxysuccinimide, and l-ethyl-3-(3-dimethylaminopropyl) carbodiimide. If desired, one or more capture reagents, such as antibodies (or fragments thereof), each of which is specific for analyte(s) can be attached to solid phases in different physical or addressable locations (e.g., such as in a biochip configuration (see, e.g., U.S. Patent No. 6,225,047; Int'l Patent App. Pub. No. WO 99/51773; U.S. Patent No. 6,329,209; Int'l Patent App. Pub. No. WO 00/56934, and U.S. Patent No. 5,242,828). If the capture reagent is attached to a mass spectrometry probe as the solid support, the amount of analyte bound to the probe can be detected by laser desorption ionization mass spectrometry. Alternatively, a single column can be packed with different beads, which are derivatized with the one or more capture reagents, thereby capturing the analyte in a single place (see, antibody-derivatized, bead-based technologies, e.g., the xMAP technology of Luminex (Austin, TX)).

After the test sample being assayed for analyte (or a fragment thereof) is brought into contact with the at least one capture antibody (for example, the first capture antibody), the mixture is incubated in order to allow for the formation of a first antibody (or multiple antibody)-analyte (or a fragment thereof) complex. The incubation can be carried out at a pH of from about 4.5 to about 10.0, at a temperature of from about 2°C to about 45°C, and for a period from at least about one (1) minute to about eighteen (18) hours, preferably from about 1 to about 24 minutes, most preferably for about 4 to about 18 minutes. The immunoassay

described herein can be conducted in one step (meaning the test sample, at least one capture antibody and at least one detection antibody are all added sequentially or simultaneously to a reaction vessel) or in more than one step, such as two steps, three steps, etc.

After formation of the (first or multiple) capture antibody/analyte (or a fragment thereof) complex, the complex is then contacted with at least one detection antibody under conditions which allow for the formation of a (first or multiple) capture antibody/analyte (or a fragment thereof)/second detection antibody complex). While captioned for clarity as the "second" antibody (e.g., second detection antibody), in fact, where multiple antibodies are used for capture and/or detection, the at least one detection antibody can be the second, third, fourth, etc. antibodies used in the immunoassay. If the capture antibody/analyte (or a fragment thereof) complex is contacted with more than one detection antibody, then a (first or multiple) capture antibody/analyte (or a fragment thereof)/(multiple) detection antibody complex is formed. As with the capture antibody (e.g., the first capture antibody), when the at least one (e.g., second and any subsequent) detection antibody is brought into contact with the capture antibody/analyte (or a fragment thereof) complex, a period of incubation under conditions similar to those described above is required for the formation of the (first or multiple) capture antibody/analyte (or a fragment thereof)/(second or multiple) detection antibody complex. Preferably, at least one detection antibody contains a detectable label. The detectable label can be bound to the at least one detection antibody (e.g., the second detection antibody) prior to, simultaneously with, or after the formation of the (first or multiple) capture antibody/analyte (or a fragment thereof)/(second or multiple) detection antibody complex. Any detectable label known in the art can be used (see discussion above, including of the Polak and Van Noorden (1997) and Haugland (1996) references).

The detectable label can be bound to the antibodies either directly or through a coupling agent. An example of a coupling agent that can be used is EDAC (l-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride), which is commercially available from Sigma-Aldrich, St. Louis, MO. Other coupling agents that can be used are known in the art. Methods for binding a detectable label to an antibody are known in the art. Additionally, many detectable labels can be purchased or synthesized that already contain end groups that facilitate the coupling of the detectable label to the antibody, such as CPSP-Acridinium Ester (i.e., 9-[N-tosyl-N-(3-carboxypropyl)]-10-(3-sulfopropyl)acridinium carboxamide) or SPSP-Acridinium Ester (i.e., N10-(3-sulfopropyl)-N-(3-sulfopropyl)-acridinium-9-carboxamide).

The (first or multiple) capture antibody/analyte/(second or multiple) detection antibody complex can be, but does not have to be, separated from the remainder of the test sample prior to quantification of the label. For example, if the at least one capture antibody (e.g., the first capture antibody) is bound to a solid support, such as a well or a bead, separation can be accomplished by removing the fluid (of the test sample) from contact with the solid support. Alternatively, if the at least first capture antibody is bound to a solid support, it can be simultaneously contacted with the analyte-containing sample and the at least one second detection antibody to form a first (multiple) antibody/analyte/second (multiple) antibody complex, followed by removal of the fluid (test sample) from contact with the solid support. If the at least one first capture antibody is not bound to a solid support, then the (first or multiple) capture antibody/analyte/(second or multiple) detection antibody complex does not have to be removed from the test sample for quantification of the amount of the label.

After formation of the labeled capture antibody/analyte/detection antibody complex (e.g., the first capture antibody/analyte/second detection antibody complex), the amount of label in the complex is quantified using techniques known in the art. For example, if an enzymatic label is used, the labeled complex is reacted with a substrate for the label that gives a quantifiable reaction such as the development of color. If the label is a radioactive label, the label is quantified using appropriate means, such as a scintillation counter. If the label is a fluorescent label, the label is quantified by stimulating the label with a light of one color (which is known as the "excitation wavelength") and detecting another color (which is known as the "emission wavelength") that is emitted by the label in response to the stimulation. If the label is a chemiluminescent label, the label is quantified by detecting the light emitted either visually or by using luminometers, x-ray film, high speed photographic film, a CCD camera, etc. Once the amount of the label in the complex has been quantified, the concentration of analyte or a fragment thereof in the test sample is determined by appropriate means, such as by use of a standard curve that has been generated using serial dilutions of analyte or a fragment thereof of known concentration. Other than using serial dilutions of analyte or a fragment thereof, the standard curve can be generated gravimetrically, by mass spectroscopy and by other techniques known in the art.

In a chemiluminescent microparticle assay employing the ARCHITECT® analyzer, the conjugate diluent pH should be about 6.0 +/- 0.2, the microparticle coating buffer should be maintained at about room temperature (i.e., at from about 17 to about 27 °C), the

microparticle coating buffer pH should be about 6.5 +/- 0.2, and the microparticle diluent pH should be about 7.8 +/- 0.2. Solids preferably are less than about 0.2%, such as less than about 0.15%, less than about 0.14%, less than about 0.13%, less than about 0.12%, or less than about 0.11%, such as about 0.10%.

FPIAs are based on competitive binding immunoassay principles. A fluorescently labeled compound, when excited by a linearly polarized light, will emit fluorescence having a degree of polarization inversely proportional to its rate of rotation. When a fluorescently labeled tracer-antibody complex is excited by a linearly polarized light, the emitted light remains highly polarized because the fluorophore is constrained from rotating between the time light is absorbed and the time light is emitted. When a "free" tracer compound (i.e., a compound that is not bound to an antibody) is excited by linearly polarized light, its rotation is much faster than the corresponding tracer-antibody conjugate produced in a competitive binding immunoassay. FPIAs are advantageous over RIAs inasmuch as there are no radioactive substances requiring special handling and disposal. In addition, FPIAs are homogeneous assays that can be easily and rapidly performed.

In view of the above, a method of determining the presence, amount, or concentration of HCV core (or a fragment thereof) in a test sample is provided. The method comprises assaying the test sample for an analyte (or a fragment thereof) by an assay (i) employing (i') at least one of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) that can bind to an analyte, and (ii') at least one detectable label and (ii) comprising comparing a signal generated by the detectable label as a direct or indirect indication of the presence, amount or concentration of analyte (or a fragment thereof) in the test sample to a signal generated as a direct or indirect indication of the presence, amount or concentration of analyte (or a fragment thereof) in a control or calibrator. The calibrator is optionally part of a series of calibrators, in which each of the calibrators differs from the other calibrators by the concentration of analyte.

The method can comprise (i) contacting the test sample with at least one first specific binding partner for HCV core (or a fragment thereof) selected from the group consisting of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) that can bind to an

analyte so as to form a first specific binding partner/analyte (or fragment thereof) complex, (ii) contacting the first specific binding partner/analyte (or fragment thereof) complex with at least one second specific binding partner for analyte (or fragment thereof) selected from the group consisting of a detectably labeled anti-analyte antibody, a detectably labeled fragment of an anti-analyte antibody that can bind to analyte, a detectably labeled variant of an anti-analyte antibody that can bind to analyte, a detectably labeled fragment of a variant of an anti-analyte antibody that can bind to analyte, and a detectably labeled DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) so as to form a first specific binding partner/analyte (or fragment thereof)/second specific binding partner complex, and (iii) determining the presence, amount or concentration of analyte in the test sample by detecting or measuring the signal generated by the detectable label in the first specific binding partner/analyte (or fragment thereof)/second specific binding partner complex formed in (ii). A method in which at least one first specific binding partner for analyte (or a fragment thereof) and/or at least one second specific binding partner for analyte (or a fragment thereof) is a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein can be preferred.

Alternatively, the method can comprise contacting the test sample with at least one first specific binding partner for HCV core (or a fragment thereof) selected from the group consisting of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) and simultaneously or sequentially, in either order, contacting the test sample with at least one second specific binding partner, which can compete with analyte (or a fragment thereof) for binding to the at least one first specific binding partner and which is selected from the group consisting of a detectably labeled analyte, a detectably labeled fragment of analyte that can bind to the first specific binding partner, a detectably labeled variant of analyte that can bind to the first specific binding partner, and a detectably labeled fragment of a variant of analyte that can bind to the first specific binding partner. Any analyte (or a fragment thereof) present in the test sample and the at least one second specific binding partner compete with each other to form a first specific binding partner/analyte (or fragment thereof) complex and a first specific binding partner/second specific binding partner complex, respectively. The method further comprises determining the presence, amount or concentration of analyte in the test sample by detecting or measuring the signal generated by the detectable label in the first

specific binding partner/second specific binding partner complex formed in (ii), wherein the signal generated by the detectable label in the first specific binding partner/second specific binding partner complex is inversely proportional to the amount or concentration of analyte in the test sample.

The above methods can further comprise diagnosing, prognosticating, or assessing the efficacy of a therapeutic/prophylactic treatment of a patient from whom the test sample was obtained. If the method further comprises assessing the efficacy of a therapeutic/prophylactic treatment of the patient from whom the test sample was obtained, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy. The method can be adapted for use in an automated system or a semi-automated system.

With regard to the methods of assay (and kit therefor), it may be possible to employ commercially available anti-analyte antibodies or methods for production of anti-analyte as described in the literature. Commercial supplies of various antibodies include, but are not limited to, Santa Cruz Biotechnology Inc. (Santa Cruz, CA), GenWay Biotech, Inc. (San Diego, CA), and R&D Systems (RDS; Minneapolis, MN).

Generally, a predetermined level can be employed as a benchmark against which to assess results obtained upon assaying a test sample for analyte or a fragment thereof, e.g., for detecting disease or risk of disease. Generally, in making such a comparison, the predetermined level is obtained by running a particular assay a sufficient number of times and under appropriate conditions such that a linkage or association of analyte presence, amount or concentration with a particular stage or endpoint of a disease, disorder or condition or with particular clinical indicia can be made. Typically, the predetermined level is obtained with assays of reference subjects (or populations of subjects). The analyte measured can include fragments thereof, degradation products thereof, and/or enzymatic cleavage products thereof.

In particular, with respect to a predetermined level as employed for monitoring disease progression and/or treatment, the amount or concentration of analyte or a fragment thereof may be "unchanged," "favorable" (or "favorably altered"), or "unfavorable" (or "unfavorably altered"). "Elevated" or "increased" refers to an amount or a concentration in a test sample that is higher than a typical or normal level or range (e.g., predetermined level), or is higher than another reference level or range (e.g., earlier or baseline sample). The term "lowered" or "reduced" refers to an amount or a concentration in a test sample that is lower than a typical or normal level or range (e.g., predetermined level), or is lower than another

reference level or range (e.g., earlier or baseline sample). The term "altered" refers to an amount or a concentration in a sample that is altered (increased or decreased) over a typical or normal level or range (e.g., predetermined level), or over another reference level or range (e.g., earlier or baseline sample).

The typical or normal level or range for analyte is defined in accordance with standard practice. Because the levels of analyte in some instances will be very low, a so-called altered level or alteration can be considered to have occurred when there is any net change as compared to the typical or normal level or range, or reference level or range, that cannot be explained by experimental error or sample variation. Thus, the level measured in a particular sample will be compared with the level or range of levels determined in similar samples from a so-called normal subject. In this context, a "normal subject" is an individual with no detectable disease, for example, and a "normal" (sometimes termed "control") patient or population is/are one(s) that exhibit(s) no detectable disease, respectively, for example. Furthermore, given that analyte is not routinely found at a high level in the majority of the human population, a "normal subject" can be considered an individual with no substantial detectable increased or elevated amount or concentration of analyte, and a "normal" (sometimes termed "control") patient or population is/are one(s) that exhibit(s) no substantial detectable increased or elevated amount or concentration of analyte. An "apparently normal subject" is one in which analyte has not yet been or currently is being assessed. The level of an analyte is said to be "elevated" when the analyte is normally undetectable (e.g., the normal level is zero, or within a range of from about 25 to about 75 percentiles of normal populations), but is detected in a test sample, as well as when the analyte is present in the test sample at a higher than normal level. Thus, inter alia, the disclosure provides a method of screening for a subject having, or at risk of having, a particular disease, disorder, or condition. The method of assay can also involve the assay of other markers and the like.

Accordingly, the methods described herein also can be used to determine whether or not a subject has or is at risk of developing a given disease, disorder or condition. Specifically, such a method can comprise the steps of:

(a) determining the concentration or amount in a test sample from a subject of analyte (or a fragment thereof) (e.g., using the methods described herein, or methods known in the art); and

(b) comparing the concentration or amount of analyte (or a fragment thereof) determined in step (a) with a predetermined level, wherein, if the concentration or amount of

analyte determined in step (a) is favorable with respect to a predetermined level, then the subject is determined not to have or be at risk for a given disease, disorder or condition. However, if the concentration or amount of analyte determined in step (a) is unfavorable with respect to the predetermined level, then the subject is determined to have or be at risk for a given disease, disorder or condition.

Additionally, provided herein is method of monitoring the progression of disease in a subject. Optimally the method comprising the steps of:

(a) determining the concentration or amount in a test sample from a subject of analyte;

(b) determining the concentration or amount in a later test sample from the subject of analyte; and

(c) comparing the concentration or amount of analyte as determined in step (b) with the concentration or amount of analyte determined in step (a), wherein if the concentration or amount determined in step (b) is unchanged or is unfavorable when compared to the concentration or amount of analyte determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened. By comparison, if the concentration or amount of analyte as determined in step (b) is favorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved.

Optionally, the method further comprises comparing the concentration or amount of analyte as determined in step (b), for example, with a predetermined level. Further, optionally the method comprises treating the subject with one or more pharmaceutical compositions for a period of time if the comparison shows that the concentration or amount of analyte as determined in step (b), for example, is unfavorably altered with respect to the predetermined level.

Still further, the methods can be used to monitor treatment in a subject receiving treatment with one or more pharmaceutical compositions. Specifically, such methods involve providing a first test sample from a subject before the subject has been administered one or more pharmaceutical compositions. Next, the concentration or amount in a first test sample from a subject of analyte is determined (e.g., using the methods described herein or as known in the art). After the concentration or amount of analyte is determined, optionally the concentration or amount of analyte is then compared with a predetermined level. If the concentration or amount of analyte as determined in the first test sample is lower than the

predetermined level, then the subject is not treated with one or more pharmaceutical compositions. However, if the concentration or amount of analyte as determined in the first test sample is higher than the predetermined level, then the subject is treated with one or more pharmaceutical compositions for a period of time. The period of time that the subject is treated with the one or more pharmaceutical compositions can be determined by one skilled in the art (for example, the period of time can be from about seven (7) days to about two years, preferably from about fourteen (14) days to about one (1) year).

During the course of treatment with the one or more pharmaceutical compositions, second and subsequent test samples are then obtained from the subject. The number of test samples and the time in which said test samples are obtained from the subject are not critical. For example, a second test sample could be obtained seven (7) days after the subject is first administered the one or more pharmaceutical compositions, a third test sample could be obtained two (2) weeks after the subject is first administered the one or more pharmaceutical compositions, a fourth test sample could be obtained three (3) weeks after the subject is first administered the one or more pharmaceutical compositions, a fifth test sample could be obtained four (4) weeks after the subject is first administered the one or more pharmaceutical compositions, etc.

After each second or subsequent test sample is obtained from the subject, the concentration or amount of analyte is determined in the second or subsequent test sample is determined (e.g., using the methods described herein or as known in the art). The concentration or amount of analyte as determined in each of the second and subsequent test samples is then compared with the concentration or amount of analyte as determined in the first test sample (e.g., the test sample that was originally optionally compared to the predetermined level). If the concentration or amount of analyte as determined in step (c) is favorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved, and the subject should continue to be administered the one or pharmaceutical compositions of step (b). However, if the concentration or amount determined in step (c) is unchanged or is unfavorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened, and the subject should be treated with a higher concentration of the one or more pharmaceutical compositions administered to the subject in step (b) or the subject should be treated with one or more pharmaceutical compositions that are different from the one or more

pharmaceutical compositions administered to the subject in step (b). Specifically, the subject can be treated with one or more pharmaceutical compositions that are different from the one or more pharmaceutical compositions that the subject had previously received to decrease or lower said subject's analyte level.

Generally, for assays in which repeat testing may be done (e.g., monitoring disease progression and/or response to treatment), a second or subsequent test sample is obtained at a period in time after the first test sample has been obtained from the subject. Specifically, a second test sample from the subject can be obtained minutes, hours, days, weeks or years after the first test sample has been obtained from the subject. For example, the second test sample can be obtained from the subject at a time period of about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years, about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years after the first test sample from the subject is obtained.

When used to monitor disease progression, the above assay can be used to monitor the progression of disease in subjects suffering from acute conditions. Acute conditions, also known as critical care conditions, refer to acute, life-threatening diseases or other critical medical conditions involving, for example, the cardiovascular system or excretory system.

Typically, critical care conditions refer to those conditions requiring acute medical intervention in a hospital-based setting (including, but not limited to, the emergency room, intensive care unit, trauma center, or other emergent care setting) or administration by a paramedic or other field-based medical personnel. For critical care conditions, repeat monitoring is generally done within a shorter time frame, namely, minutes, hours or days (e.g., about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days or about 7 days), and the initial assay likewise is generally done within a shorter timeframe, e.g., about minutes, hours or days of the onset of the disease or condition.

The assays also can be used to monitor the progression of disease in subjects suffering from chronic or non-acute conditions. Non-critical care or, non-acute conditions, refers to conditions other than acute, life-threatening disease or other critical medical conditions involving, for example, the cardiovascular system and/or excretory system. Typically, non-acute conditions include those of longer-term or chronic duration. For non-acute conditions, repeat monitoring generally is done with a longer timeframe, e.g., hours, days, weeks, months or years (e.g., about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks,

about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years, about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years), and the initial assay likewise generally is done within a longer time frame, e.g., about hours, days, months or years of the onset of the disease or condition.

Furthermore, the above assays can be performed using a first test sample obtained from a subject where the first test sample is obtained from one source, such as urine, serum or plasma. Optionally, the above assays can then be repeated using a second test sample obtained from the subject where the second test sample is obtained from another source. For example, if the first test sample was obtained from urine, the second test sample can be obtained from serum or plasma. The results obtained from the assays using the first test sample and the second test sample can be compared. The comparison can be used to assess the status of a disease or condition in the subject.

Moreover, the present disclosure also relates to methods of determining whether a subject predisposed to or suffering from a given disease, disorder or condition will benefit from treatment. In particular, the disclosure relates to analyte companion diagnostic methods and products. Thus, the method of "monitoring the treatment of disease in a subject" as described herein further optimally also can encompass selecting or identifying candidates for therapy.

Thus, in particular embodiments, the disclosure also provides a method of determining whether a subject having, or at risk for, a given disease, disorder or condition is a candidate for therapy. Generally, the subject is one who has experienced some symptom of a given disease, disorder or condition or who has actually been diagnosed as having, or being at risk for, a given disease, disorder or condition, and/or who demonstrates an unfavorable concentration or amount of analyte or a fragment thereof, as described herein.

The method optionally comprises an assay as described herein, where analyte is assessed before and following treatment of a subject with one or more pharmaceutical compositions (e.g., particularly with a pharmaceutical related to a mechanism of action involving analyte), with immunosuppressive therapy, or by immunoabsorption therapy, or where analyte is assessed following such treatment and the concentration or the amount of analyte is compared against a predetermined level. An unfavorable concentration of amount of analyte observed following treatment confirms that the subject will not benefit from

receiving further or continued treatment, whereas a favorable concentration or amount of analyte observed following treatment confirms that the subject will benefit from receiving further or continued treatment. This confirmation assists with management of clinical studies, and provision of improved patient care.

It goes without saying that, while certain embodiments herein are advantageous when employed to assess a given disease, disorder or condition as discussed herein, the assays and kits can be employed to assess analyte in other diseases, disorders and conditions. The method of assay can also involve the assay of other markers and the like.

The method of assay also can be used to identify a compound that ameliorates a given disease, disorder or condition. For example, a cell that expresses analyte can be contacted with a candidate compound. The level of expression of analyte in the cell contacted with the compound can be compared to that in a control cell using the method of assay described herein.

In yet another detection method, each of the binding proteins as described herein can be employed in the detection of HCV antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis.

In addition, these binding proteins can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific HCV proteins from cell cultures, or biological tissues such as blood and liver.

The monoclonal antibodies as described herein can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect HCV core antigens. It is contemplated that combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one anti-HCV core antibody as described herein with antibodies to other HCV regions, each having different binding specificities. Thus, this cocktail can include the monoclonal antibodies as described herein, which are directed to HCV core protein, and other monoclonal antibodies to other antigenic determinants of the HCV genome. Examples of other monoclonal antibodies useful for these contemplated cocktails include those to HCV C-100, HCV 33C, HCV CORE, HCV NS5 and/or HCV putative ENV, which are disclosed in, for example, U.S. Ser. No. 07/610,175 entitled MONOCLONAL ANTIBODIES TO HEPATITIS C VIRUS AND METHOD FOR USING SAME, U.S.S.N. 07/610,175 entitled MONOCLONAL ANTIBODIES TO HCV 33C

PROTEINS AND METHODS FOR USING SAME, U.S. Ser. No. 07/648,475 entitled MONOCLONAL ANTIBODIES TO PUTATIVE HCV ENVELOPE REGION AND METHODS FOR USING SAME, U.S. Ser. No. 07/648,473 entitled MONOCLONAL ANTIBODIES TO HCV CORE PROTEINS AND METHODS FOR USING SAME and in co-filed patent application entitled MONOCLONAL ANTIBODIES TO HCV NS5 PROTEIN AND METHODS FOR USING SAME, U.S. Ser. No. 07/748,563, all of which enjoy common ownership and are incorporated herein by reference. This cocktail of monoclonal antibodies as described herein would be used in the assay formats detailed herein in place of the monoclonal antibody to HCV core, and thus would be able to detect the HCV core and other HCV antigens.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to HCV core or other HCV proteins used in the assay, such as HCV C-100 protein, HCV 33C protein, HCV ENV, HCV E2/NS1 or HCV NS5 protein. The polyclonal antibody used preferably is of mammalian origin; human, goat, rabbit or sheep anti-HCV polyclonal antibody can be used. Most preferably, the polyclonal antibody is rabbit polyclonal anti-HCV antibody. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different HCV specificity, they would be useful for diagnosis, evaluation and prognosis of HCV infection, as well as for studying HCV protein differentiation and specificity.

Test samples which can be tested by the methods as described herein described herein include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, biological fluids such as cell culture supernatants, fixed tissue specimens and fixed ceil specimens.

The indicator reagent comprises a signal-generating compound (label) that is capable of generating a measurable signal detectable by external means conjugated (attached) to a specific binding member for HCV core. "Specific binding member" as used herein means a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to being an antibody member of a specific binding pair for HCV core, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor

and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to HCV core as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay.

The various signal generating compounds (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as acridinium, phenanthridinium and dioxetane compounds, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies as described herein are easily adaptable. In scanning probe microscopy, in particular in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies as described herein, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunnelling microscopy eliminates the need for labels that normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. Such a system is described in pending U.S. patent application Ser. No. 662,147, which enjoys common ownership and is incorporated herein by reference.

The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (the analyte specific substance, which is the monoclonal antibody as described herein) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece, which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Activated silane compounds such as triethoxy amino propyl silane (available from Sigma Chemical Co., St. Louis, Mo.), triethoxy vinyl silane

(Aldrich Chemical Co., Milwaukee, Wis.), and (3-mercapto-propyl)trimethoxy silane (Sigma Chemical Co., St. Louis, Mo.) can be used to introduce reactive groups such as amino-, vinyl, and thiol, respectively. Such activated surfaces can be used to link the binding partner directly (in the cases of amino or thiol) or the activated surface can be further reacted with linkers such as glutaraldehyde, bis(succinimidyl) suberate, SPPD 9 succinimidyl 3-[2-pyridyldithio] propionate), SMCC (succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxylate), SIAB (succinimidyl [4-iodoacetyl] aminobenzoate), and SMPB (succinimidyl 4-[l-maleimidophenyl] butyrate) to separate the binding partner from the surface. The vinyl group can be oxidized to provide a means for covalent attachment. It also can be used as an anchor for the polymerization of various polymers such as poly acrylic acid, which can provide multiple attachment points for specific binding partners. The amino surface can be reacted with oxidized dextrans of various molecular weights to provide hydrophilic linkers of different size and capacity. Examples of oxidizable dextrans include Dextran T-40 (molecular weight 40,000 daltons), Dextran T-l 10 (molecular weight 110,000 daltons), Dextran T-500 (molecular weight 500,000 daltons), Dextran T-2M (molecular weight 2,000,000 daltons) (all of which are available from Pharmacia, Piscataway, N.J.), or Ficoll (molecular weight 70,000 daltons (available from Sigma Chemical Co., St. Louis, Mo.). Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries described by pending U.S. patent application Ser. Nos. 150,278, filed Jan. 29, 1988, and Ser. No. 375,029, filed Jul. 7, 1989, each of which enjoys common ownership and each of which is incorporated herein by reference. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present disclosure expresses a preference for the use of solid phases, it is contemplated that the monoclonal antibodies as described herein can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the disclosure.

It is contemplated that the reagent employed for the assay can be provided in the form of a kit with one or more containers such as vials or bottles, with each container containing a

separate reagent such as a monoclonal antibody, or a cocktail of monoclonal antibodies, detection reagents and washing reagents employed in the assay.

The antibodies can also be used as a means of enhancing the immune response. The antibodies can be administered in amount similar to those used for other therapeutic administrations of antibody. For example, normal immune globulin is administered at 0.02 0.1 ml/lb body weight during the early incubation period of other viral diseases such as rabies, measles, and hepatitis B to interfere with viral entry into cells. Thus, antibodies reactive with the HCV core proteins can be passively administered alone or in conjunction with another anti-viral agent to a host infected with an HCV to enhance the immune response and/or the effectiveness of an antiviral drug.

When used as a means of inducing anti-HCV virus antibodies in an animal, the manner of injecting the antibody is the same as for vaccination purposes, namely intramuscularly, intraperitoneally, subcutaneous ly or the like in an effective concentration in a physiologically suitable diluent with or without adjuvant. One or more booster injections may be desirable.

The present disclosure also relates to the use of single-stranded antisense poly- or oligonucleotides, or short double-stranded oligonucleotides such as siRNA's or shRNA's derived from nucleotide sequences substantially homologous to those shown in SEQ ID NO: 13 through 24, or any portion thereof of about 19-50 nt's in length, to inhibit the expression of HCV core genes. Substantially homologous refers to a level of homology between a nucleic acid sequence and the sequence of the referenced SEQ ID NO. Preferably, the level of homology is in excess of 80%, more preferably in excess of 90%, with a preferred nucleic acid sequence being in excess of 95% homologous with the DNA sequence shown in the indicated SEQ ID NO. These anti-sense poly- or oligonucleotides can be either DNA or RNA. The targeted sequence is typically messenger RNA and more preferably, a single sequence required for processing or translation of the RNA. The anti-sense poly- or oligonucleotides can be conjugated to a polycation such as polylysine as disclosed in Lemaitre, M. et al. ((1989) Proc. Natl. Acad. Sci. USA 84:648 652) and this conjugate can be administrated to a mammal in an amount sufficient to hybridize to and inhibit the function of the messenger RNA.

The present disclosure further relates to multiple computer-generated alignments of the nucleotide and deduced amino acid sequences shown in SEQ ID NO: 1 through SEQ ID NO: 20. Computer analysis of the nucleotide sequences shown in SEQ ID NO: 11 through 20

and of the deduced amino acid sequences shown in SEQ ID NO: 1 through SEQ ID NO: 10 can be carried out using commercially available computer programs known to one skilled in the art.

F. Kits

A kit for assaying a test sample for the presence, amount or concentration of HCV core protein (or a fragment thereof) in a test sample is also provided. The kit comprises at least one component for assaying the test sample for HCV core protein (or a fragment thereof) and instructions for assaying the test sample for the analyte (or a fragment thereof). The at least one component for assaying the test sample for the analyte (or a fragment thereof) can include a composition comprising an anti-HCV core protein monoclonal antibody or an anti-HCV core protein DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), which is optionally immobilized or capable of being immobilized on a solid phase.

The kit can comprise at least one component for assaying the test sample for HCV core protein by immunoassay, e.g., chemiluminescent microparticle immunoassay, and instructions for assaying the test sample for an analyte by immunoassay, e.g., chemiluminescent microparticle immunoassay. For example, the kit can comprise at least one specific binding partner for an analyte, such as an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof that can bind to the analyte, a variant thereof that can bind to the analyte, or a fragment of a variant that can bind to the analyte) or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), either of which can be detectably labeled. Alternatively or additionally, the kit can comprise detectably labeled analyte (or a fragment thereof that can bind to an anti-analyte, monoclonal/polyclonal antibody or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof)), which can compete with any analyte in a test sample for binding to an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof that can bind to the analyte, a variant thereof that can bind to the analyte, or a fragment of a variant that can bind to the analyte) or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), either of which can be immobilized on a solid support. The kit can comprise a calibrator or control, e.g., isolated or purified analyte. The kit can comprise at least one container (e.g., tube, microtiter plates or strips, which can be already coated with a first specific binding partner, for example) for conducting the assay, and/or a buffer, such as an assay buffer or a wash

buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (e.g., an enzymatic label), or a stop solution. Preferably, the kit comprises all components, i.e., reagents, standards, buffers, diluents, etc., which are necessary to perform the assay. The instructions can be in paper form or computer-readable form, such as a disk, CD, DVD, or the like.

Any antibodies, such as an anti-analyte antibody or an anti-analyte DVD-Ig, or tracer can incorporate a detectable label as described herein, such as a fluorophore, a radioactive moiety, an enzyme, a biotin/avidin label, a chromophore, a chemiluminescent label, or the like, or the kit can include reagents for carrying out detectable labeling. The antibodies, calibrators and/or controls can be provided in separate containers or pre-dispensed into an appropriate assay format, for example, into microtiter plates.

Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays.

The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, enzyme substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. The kit can additionally include one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

The various components of the kit optionally are provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing a sample (e.g., a container or cartridge for a urine sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instruments for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

If the detectable label is at least one acridinium compound, the kit can comprise at least one acridinium-9-carboxamide, at least one acridinium-9-carboxylate aryl ester, or any

combination thereof. If the detectable label is at least one acridinium compound, the kit also can comprise a source of hydrogen peroxide, such as a buffer, a solution, and/or at least one basic solution. If desired, the kit can contain a solid phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc or chip.

The kit (or components thereof), as well as the method of determining the presence, amount or concentration of an analyte in a test sample by an assay, such as an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, e.g., in U.S. Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, IL) as ARCHITECT®.

Some of the differences between an automated or semi-automated system as compared to a non-automated system (e.g., ELISA) include the substrate to which the first specific binding partner (e.g., an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) or an anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) is attached; either way, sandwich formation and analyte reactivity can be impacted), and the length and timing of the capture, detection and/or any optional wash steps. Whereas a non-automated format, such as an ELISA, may require a relatively longer incubation time with sample and capture reagent (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT®, Abbott Laboratories) may have a relatively shorter incubation time (e.g., approximately 18 minutes for ARCHITECT®). Similarly, whereas a non-automated format, such as an ELISA, may incubate a detection antibody, such as the conjugate reagent, for a relatively longer incubation time (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT®) may have a relatively shorter incubation time (e.g., approximately 4 minutes for the ARCHITECT®).

Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, e.g., U.S. Patent No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich

immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Patent No. 5,063,081, U.S. Patent App. Pub. No. 2003/0170881, U.S. Patent App. Pub. No. 2004/0018577, U.S. Patent App. Pub. No. 2005/0054078, and U.S. Patent App. Pub. No. 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

In particular, with regard to the adaptation of an analyte assay to the I-STAT® system, the following configuration is preferred. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) or anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof), are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an I-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the wall of the sample-holding chamber of the cartridge there is a layer comprising a specific binding partner for an analyte, such as an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind the analyte) or an anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind the analyte), either of which can be detectably labeled. Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

In operation, a sample suspected of containing an analyte is added to the holding chamber of the test cartridge, and the cartridge is inserted into the I-STAT® reader. After the specific binding partner for an analyte has dissolved into the sample, a pump element within the cartridge forces the sample into a conduit containing the chip. Here it is oscillated to promote formation of the sandwich. In the penultimate step of the assay, fluid is forced out of the pouch and into the conduit to wash the sample off the chip and into a waste chamber. In the final step of the assay, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of analyte in the sample by means of an embedded algorithm and factory-determined calibration curve.

It further goes without saying that the methods and kits as described herein necessarily encompass other reagents and methods for carrying out the immunoassay. For

instance, encompassed are various buffers such as are known in the art and/or which can be readily prepared or optimized to be employed, e.g., for washing, as a conjugate diluent, microparticle diluent, and/or as a calibrator diluent. An exemplary conjugate diluent is ARCHITECT® conjugate diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL) and containing 2-(N-morpholino)ethanesulfonic acid (MES), a salt, a protein blocker, an antimicrobial agent, and a detergent. An exemplary calibrator diluent is ARCHITECT® human calibrator diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL), which comprises a buffer containing MES, other salt, a protein blocker, and an antimicrobial agent. Additionally, as described in U.S. Patent Application No. 61/142,048 filed December 31, 2008, improved signal generation may be obtained, e.g., in an I-Stat cartridge format, using a nucleic acid sequence linked to the signal antibody as a signal amplifier.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLES

Example 1: Deduced Amino Acid Sequences of heavy and light chain variable domains of Anti-
HCV core monoclonal antibodies

Hybridoma cloning and growth of cells: Details of the cloning process have been reported previously (Katsumi Aoyagi et al, J Clin Microbiol. 1999 June; 37(6): 1802-1 808). Two rounds of cloning via limiting dilution and two rounds of cloning using the Quixell Cell Transfer System (Stoelting Co., Wood Dale, IL) were performed before formation of a Master Cell Bank. Cells from this master cell bank were analyzed for population homogeneity after micromanipulation with a Quixell Cell Transfer System. Single cells were seeded into each well of three 96-well tissue culture plates. Plates were incubated in a 35-37 °C humid incubator with 4-6% C02. Wells with growing cells were marked then passed into 24- well plates for further growth. The supernatants from the 24-well plate wells were

quantified for IgG. The ratio of IgG nonproducing cells to producing cells was used as a measure of clonality. Cells used for sequencing were grown in a propriety cell culture medium based on Hybridoma Serum Free Medium (HSFM) manufactured by Invitrogen (Grand Island, NY). Cells were seeded into cell culture flasks at a density of lxlO^5 viable cells/ml and maintained in a C02 incubator at 36°C and 5% C02. Cells were harvested in log phase growth with viability greater than 80%.

To obtain nucleotide sequences of the heavy and light chain variable domains of anti-HCV core monoclonals AOT-3, CI 1-3, CI 1-7, CI 1-9, and CI 1-14, mRNA was isolated from hybridoma cells grown in cell culture. The RNA was reverse transcribed and amplified using RT-PCR. Oligonucleotide primers used for cDNA synthesis and subsequent amplification were from Novagen (cat no. 6983 1). Amplified cDNA was isolated by agarose gel electrophoresis, excised and purified from the agarose gel and sequenced using standard methods. Variable region nucleotide sequences for each antibody are listed in Table 2 (SEQ ID NO: 11 through SEQ ID NO: 20).

Example 2: Generation of Dual Variable Domain Immunoglobulin (DVD-Ig)

The anti-HCV core DVD-Ig binding protein is designed such that two different light chain variable domains (VL) from the two different parent mAbs are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem directly or via a linker, followed by the constant domain CHI and Fc region (FIG.l).

Example 3: Assays Used to Identify and Characterize Parent Antibodies and DVD-Ig

A Direct Bind ELISA can be used to identify and characterize parent antibodies and DVD-Ig binding proteins. Enzyme Linked Immunosorbent Assays to screen for antibodies that bind HCV core protein are performed as follows. High bind ELISA plates (Corning Costar # 3369, Acton, MA) are coated with IOOμL/weII of 10μg/ml of HCV core protein in phosphate buffered saline (PBS) overnight at 4°C. Plates are washed four times with PBS containing 0.02% Tween 20. Plates are blocked by the addition of 300 μL/weII blocking solution (non-fat dry milk powder, various retail suppliers, diluted to 2% in PBS) for 1/2 hour

at room temperature. Plates are washed four times after blocking with PBS containing 0.02% Tween 20.

Alternatively, one hundred microliters per well of 10 μg/ml of Histidine (His) tagged desired HCV core protein (R&D Systems, Minneapolis, MN) are added to ELISA plates coated with monoclonal mouse anti-polyHistidine antibody as described above and incubated for 1 hour at room temperature. Wells are washed four times with PBS containing 0.02% Tween 20.

One hundred microliters of antibody preparations diluted in blocking solution as described above are added to the desired HCV core protein plate or desired target antigen / FC fusion plate or the anti-polyHistidine antibody / His tagged desired target antigen plate prepared as described above and incubated for 1 hour at room temperature. Wells are washed four times with PBS containing 0.02% Tween 20.

One hundred microliters of lOng/mL goat anti-human IgG -FC specific HRP conjugated antibody (Southern Biotech # 2040-05, Birmingham, AL) are added to each well of the desired target antigen plate or anti-polyHistidine antibody / Histidine tagged desired target antigen plate. Alternatively, one hundred microliters of 10 ng/mL goat anti-human IgG -kappa light chain specific HRP conjugated antibody (Southern Biotech # 2060-05 Birmingham, AL) are added to each well of the desired target antigen / FC fusion plate and incubated for 1 hour at room temperature. Plates are washed 4 times with PBS containing 0.02% Tween 20.

One hundred microliters of enhanced TMB solution (Neogen Corp. #308177, K Blue, Lexington, KY) are added to each well and incubated for 10 minutes at room temperature. The reaction was stopped by the addition of 50 μL IN sulphuric acid. Plates are read spectrophotometrically at a wavelength of 450 nm.

Example 4: Anti-HCV core DVD-Ig Binding Proteins

Table 3 lists anti-HCV core DVD-Ig's that were prepared using the deduced amino acid sequences of the heavy and light chain domains of anti-HCV monoclonal antibodies as disclosed herein (SEQ ID NO: 1 through SEQ ID NO: 10; see Table 1), and linker sequences as disclosed herein.

Table 3:

Sequence Name	Sequence
ADD60. DVD Ig HCV	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL

Sequence Name	Sequence
Cll-9 (out) and HCV Cll-14 (in) Short linker VH (SEQ ID:48)	AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS ASTKGP QVQLQQPGAE LVMPGASVKM SCKASGYTFT DYWMHWVKQR PGQGLEWIGA IDTSDSQTDY NQKYRGKATL TVDESASTAY MQLNSLTSED SAVYYCATIY YDYDGSFDQW GQGTTLTVSS
VL (SEQ ID: 49)	QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR TVAAPSVFIFPP DTVMTQSQKF MSTSVGDRVS ITCKASQNVR TVVAWYQQKP GQSPKALIYL ASNRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCLQ HWNYPLTFGG GTKLEIKR
ADD61. DVD Ig HCV Cll-9 (out) and HCV Cll-14 (in) Long linker VH (SEQ ID: 50)	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS ASTKGPSVFPLAP QVQLQQPGAE LVMPGASVKM SCKASGYTFT DYWMHWVKQR PGQGLEWIGA IDTSDSQTDY NQKYRGKATL TVDESASTAY MQLNSLTSED SAVYYCATIY YDYDGSFDQW GQGTTLTVSS
VL (SEQ ID: 51)	QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR TVAAP DTVMTQSQKF MSTSVGDRVS ITCKASQNVR TVVAWYQQKP GQSPKALIYL ASNRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCLQ HWNYPLTFGG GTKLEIKR
ADD62. DVD Ig HCV Cll-14 (out) and HCV Cll-9 (in) Short linker VH (SEQ ID: 52)	QVQLQQPGAE LVMPGASVKM SCKASGYTFT DYWMHWVKQR PGQGLEWIGA IDTSDSQTDY NQKYRGKATL TVDESASTAY MQLNSLTSED SAVYYCATIY YDYDGSFDQW GQGTTLTVSS ASTKGP QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS
VL (SEQ ID: 53)	DTVMTQSQKF MSTSVGDRVS ITCKASQNVR TVVAWYQQKP GQSPKALIYL ASNRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCLQ HWNYPLTFGG GTKLEIKR

Sequence Name	Sequence
	TVAAP QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR
ADD63. DVD Ig HCV Cll-14 (out) and HCV Cll-9 (in) Long linker VH (SEQ ID: 54)	QVQLQQPGAE LVMPGASVKM SCKASGYTFT DYWMHWVKQR PGQGLEWIGA IDTSDSQTDY NQKYRGKATL TVDESASTAY MQLNSLTSED SAVYYCATIY YDYDGSFDQW GQGTTLTVSS ASTKGPSVFPLAP QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS
VL (SEQ ID: 55)	DTVMTQSQKF MSTSVGDRVS ITCKASQNVR TVVAWYQQKP GQSPKALIYL ASNRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCLQ HWNYPLTFGG GTKLEIKR TVAAPSVFIFPP QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR
ADD64 . DVD Ig HCV Cll-7 (out) and HCV AOT3 (in) Short linker VH (SEQ ID: 56)	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS ASTKGP QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS
VL (SEQ ID: 57)	DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR TVAAP DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR
ADD65. DVD Ig HCV Cll-7 (out) and HCV AOT3 (in) Long	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS ASTKGPSVFPLAP

Sequence Name	Sequence
linker VH (SEQ ID: 58)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS
VL (SEQ ID: 59)	DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR TVAAPSVFIFPP DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR
ADD66. DVD Ig HCV AOT3 (out) and HCV Cll-7 (in) Short linker VH (SEQ ID: 60)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS ASTKGP QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS
VL (SEQ ID: 61)	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR TVAAP DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR
ADD67. DVD Ig HCV AOT3 (out) and HCV Cll-7 (in) Long linker VH (SEQ ID: 62)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS ASTKGPSVFPLAP QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS
VL (SEQ ID: 63)	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR TVAAPSVFIFPP DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK

Sequence Name	Sequence
	LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR
ADD68. DVD Ig HCV Cll-9 (out) and HCV AOT3 (in) Short linker VH (SEQ ID: 64)	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS ASTKGP QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS
VL (SEQ ID: 65)	QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR TVAAP DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR
ADD69. DVD Ig HCV Cll-9 (out) and HCV AOT3 (in) Long linker VH (SEQ ID: 66)	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS ASTKGPSVFPLAP QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS
VL (SEQ ID: 67)	QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR TVAAPSVFIFPP DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR
ADD70. DVD Ig HCV AOT3 (out) and HCV Cll-9 (in) Short linker VH (SEQ ID: 68)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS ASTKGP QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS

Sequence Name	Sequence
VL (SEQ ID: 69)	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR TVAAP QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR
ADD71. DVD Ig HCV AOT3 (out) and HCV Cll-9 (in) Long linker VH (SEQ ID: 70)	QIQLVQSGPE LKKPGETVKI SCKASGYSFT DYSMHWMKQT PGKGLKWMGW INTETGQPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCALWD YWGQGTTLTV SS ASTKGPSVFPLAP QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TYGIGVGWIR QPSGKGLEWL AHIWWNDDNY YNTALKSRLT ISKDTSNNQV FLKIASVDTV DTATYYCVRI GGPAMDYWGQ GTSVTVSS
VL (SEQ ID: 71)	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSDGITYLYW FLQKPGQSPQ LLIYQMPNVA SGVPDRFSSS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP PTFGGGTKLE IRR TVAAPSVFIFPP QIVLTPSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYNT STLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPYTFGGG TKLEIKR
ADD72. DVD Ig HCV Cll-3 (out) and HCV Cll-7 (in) Short linker VH (SEQ ID: 72)	QVQLQQPGAE LVKPGTSVKL SCKASGYTFT SYWMHWVKLR PGQGFEWIGE INPRYGDTSY NERFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYCSTDD YDRGFAYWGQ GTLVTVSA ASTKGP QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS
VL (SEQ ID: 73)	DVVMTQTPLT LSVTIGQPAS ISCKSSQSLL YSNGKNYLNW LFQRPGQSPK RLIYLVSKLD SGVPDRFTGS GSGTDFTLRI SRVEAEDLGV YYCVQGTHFP YTFGGGTKLE IKR TVAAP DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLRI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR
ADD73. DVD Ig HCV Cll-3 (out) and HCV Cll-7 (in) Long	QVQLQQPGAE LVKPGTSVKL SCKASGYTFT SYWMHWVKLR PGQGFEWIGE INPRYGDTSY NERFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYCSTDD YDRGFAYWGQ GTLVTVSA ASTKGPSVFPLAP

Sequence Name	Sequence
linker VH (SEQ ID: 74)	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS
VL (SEQ ID: 75)	DVVMTQTPLT LSVTIGQPAS ISCKSSQSLL YSNGKNYLNW LFQRPGQSPK RLIYLVSKLD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCVQGTHFP YTFGGGTKLE IKR TVAAPSVFIFPP DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR
ADD74 . DVD Ig HCV Cll-7 (out) and HCV Cll-3 (in) Short linker VH (SEQ ID: 76)	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS ASTKGP QVQLQQPGAE LVKPGTSVKL SCKASGYTFT SYWMHWVKLR PGQGFEWIGE INPRYGDTSY NERFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYCSTDD YDRGFAYWGQ GTLVTVSA
VL (SEQ ID: 77)	DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR TVAAP DVVMTQTPLT LSVTIGQPAS ISCKSSQSLL YSNGKNYLNW LFQRPGQSPK RLIYLVSKLD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCVQGTHFP YTFGGGTKLE IKR
ADD75. DVD Ig HCV Cll-7 (out) and HCV Cll-3 (in) Long linker VH (SEQ ID: 78)	QLQQFGAELV KPGASVKISC KASGYTFTDY NMDWVKQSLG KSLEWIGDIN PNDDSTNYNQ KFKGKATLTV DKSSRTAYME LRSLTSEDTA VYYCAREGYW YFDVWGAGTT VTVSS ASTKGPSVFPLAP QVQLQQPGAE LVKPGTSVKL SCKASGYTFT SYWMHWVKLR PGQGFEWIGE INPRYGDTSY NERFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYCSTDD YDRGFAYWGQ GTLVTVSA
VL (SEQ ID:79)	DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK LLIYKVSKRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IKR TVAAPSVFIFPP DVVMTQTPLT LSVTIGQPAS ISCKSSQSLL YSNGKNYLNW LFQRPGQSPK RLIYLVSKLD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCVQGTHFP YTFGGGTKLE IKR

The present disclosure incorporates by reference in their entirety techniques well known in the field of molecular biology and drug delivery. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel et al. (eds.), Current Protocols in Molecular Biology. John Wiley & Sons, NY (1993);

Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999), John Wiley & Sons, NY. (ISBN 0-471-32938-X);

Controlled Drug Bioavailability. Drug Product Design and Performance. Smolen and Ball (eds.), Wiley, New York (1984);

Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 201-16, Oxford University Press, New York, New York, (1999);

Goodson, in Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984);

Hammerling, et al, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981);

Harlow et al., Antibodies: A Laboratory Manual. (Cold Spring Harbor Laboratory Press, 2nd ed. 1988);

Kabat et al, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991);

Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest. Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242;

Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5);

Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990);

Lu and Weiner eds., Cloning and Expression Vectors for Gene Function Analysis (2001) BioTechniques Press. Westborough, MA. 298 pp. (ISBN 1-881299-21-X);

Medical Applications of Controlled Release. Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974);

Old, R.W. & S.B. Primrose, Principles of Gene Manipulation: An Introduction To Genetic Engineering (3d Ed. 1985) Blackwell Scientific Publications, Boston. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4);

Sambrook, J. et al. eds., Molecular Cloning: A Laboratory Manual (2d Ed. 1989) Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6);

Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978; and

Winnacker, E.L. From Genes To Clones: Introduction To Gene Technology (1987) VCH Publishers, NY (translated by Horst Ibelgaufts). 634 pp. (ISBN 0-89573-614-4).

The contents of all cited references (including catalogs, literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety, as are the references cited therein. The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

The present disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the present disclosure described herein. Scope of the present disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

WHAT IS CLAIMED IS:

1. A monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the heavy chain of the monoclonal antibody comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

2. A monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the light chain of the monoclonal antibody comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

3. A monoclonal antibody according to claim 1 or 2, wherein the variable region of the heavy chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 1, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 2.

4. A monoclonal antibody according to claim 1 or 2, wherein the variable region of the heavy chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 3, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 4.

5. A monoclonal antibody according to claim 1 or 2, wherein the variable region of the heavy chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 5, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 6.

6. A monoclonal antibody according to claim 1 or 2, wherein the variable region of the heavy chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 7, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 8.

7. A monoclonal antibody according to claim 1 or 2, wherein the variable region of the heavy chain comprises an amino acid having at least 90% identity with SEQ ID NO: 9, and the variable region of the light chain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 10.

8. The monoclonal antibody according to any one of claims 1-7, wherein the monoclonal antibody is a human or a chimeric antibody.

9. An isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes a monoclonal antibody according to claim 1 or 2.

10. The isolated nucleic acid molecule of claim 9 comprising RNA.

11. The isolated nucleic acid molecule of claim 9 comprising DNA.

12. An isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 11, 13, 15, 17, and 19.

13. An isolated nucleic acid molecule comprising or complementary to a nucleic acid sequence that encodes a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, wherein the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 12, 14, 16, 18 and 20.

14. The isolated nucleic acid molecule of claim 12 or 13, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 11, and the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 12.

15. The isolated nucleic acid molecule of claim 12 or 13, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 13, and the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 14.

16. The isolated nucleic acid molecule of claim 12 or 13, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 15, and the variable region of the light chain

of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 16.

17. The isolated nucleic acid molecule of claim 12 or 13, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 17, and the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 18.

18. The isolated nucleic acid molecule of claim 12 or 13, wherein the variable region of the heavy chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 19, and the variable region of the light chain of the monoclonal antibody is encoded by a nucleotide sequence having at least 90% identity with SEQ ID NO: 20.

19. An isolated recombinant vector comprising the nucleic acid molecule of any one of claims 12-18.

20. A host cell comprising the recombinant vector of claim 19.

21. A pharmaceutical or diagnostic composition comprising a monoclonal antibody according to claim 1 or 2 and a pharmaceutically acceptable carrier, diluent or excipient.

22. A pharmaceutical or diagnostic composition according to claim 21 comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a DNA sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 11, 13, 15, 17 and 19, and a pharmaceutically acceptable carrier.

23. A pharmaceutical or diagnostic composition according to claim 21 comprising a monoclonal antibody that specifically binds hepatitis C core protein, wherein the monoclonal antibody comprises a light chain variable region encoded by a DNA sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 12, 14, 16, 18, and 20, and a pharmaceutically acceptable carrier.

24. A pharmaceutical or diagnostic composition according to claim 22 or 23, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a

DNA sequence having at least 90% identity with SEQ ID NO: 11 and a light chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 12.

25. A pharmaceutical or diagnostic composition according to claim 22 or 23, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 13 and a light chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 14.

26. A pharmaceutical or diagnostic composition according to claim 22 or 23, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 15 and a light chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 16.

27. A pharmaceutical or diagnostic composition according to claim 22 or 23, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a DNA sequence having at least 90% identity with of SEQ ID NO: 17 and a light chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 18.

28. A pharmaceutical or diagnostic composition according to claim 22 or 23, wherein the monoclonal antibody comprises a heavy chain variable region encoded by a DNA sequence having at least 90% identity with of SEQ ID NO: 19 and a light chain variable region encoded by a DNA sequence having at least 90% identity with SEQ ID NO: 20.

29. A method of reducing or eliminating human hepatitis C virus activity in a human subject, comprising administering to a subject in need thereof an effective amount of a humanized monoclonal antibody of claim 1 or 2.

30. A method of detecting the presence of human hepatitis C virus in a sample, the method comprising contacting the sample with a humanized monoclonal antibody of claim 1 or 2.

31. An immunoassay for determining the presence or amount of human hepatitis C virus in a sample, comprising: providing a reagent comprising a humanized monoclonal antibody according to claim 1 or 2; combining the monoclonal antibody reagent with the sample for a time sufficient for the monoclonal antibody reagent to bind to any human hepatitis C virus in the sample; and determining the presence or amount of human hepatitis C

virus present in the sample based on specific binding of the monoclonal antibody reagent to the human hepatitis C virus core protein.

32. The immunoassay method according to claim 31, wherein the monoclonal antibody reagent is immobilized or is capable of being immobilized on a solid support.

33. The immunoassay according to claim 31, wherein the monoclonal antibody reagent is labeled with a detectable label.

34. The immunoassay method according to claim 31, further comprising:

a. providing a reagent comprising a binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, XI is a linker with the proviso that it is not CHI, and X2 is an Fc region; and two polypeptide chains comprise VDl-(Xl)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, XI is a linker with the proviso that it is not CHI, and X2 does not comprise an Fc region; and n is 0 or 1; wherein said four polypeptide chains of said binding protein form four functional antigen binding sites, wherein at least one heavy chain variable domain an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9, and at least one light chain variable domain comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10; and

b. combining the binding protein reagent of (a) with the sample for a time sufficient for the binding protein reagent to bind to any human hepatitis C virus in the sample.

35. The immunoassay method according to claim 34, wherein the binding protein reagent is immobilized or is capable of being immobilized on a solid support.

36. An immunoassay device for detecting the presence or absence of human hepatitis C virus in a sample, the device comprising: a humanized monoclonal antibody of claim 1 or 2 immobilized on a solid support.

37. A kit for detecting the presence or absence of human hepatitis C virus in a sample, the kit comprising: the humanized monoclonal antibody of claim 1 or 2; and instructions for determining whether the sample contains human hepatitis C virus.

38. The kit according to claim 37, wherein the humanized monoclonal antibody is immobilized on a solid support.

39. A binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD_1-$(X_1)_n$-VD_2-C-p$\zeta_2)_n$, wherein VDi is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, Xi is a linker with the proviso that it is not CHi, and X2 is an Fc region; and two polypeptide chains comprise VD_1-$(X_1)_n$-VD_2-C-p$\zeta_2)_n$, wherein VDi is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, Xi is a linker with the proviso that it is not CHi, and X2 does not comprise an Fc region; and n is 0 or 1; wherein said four polypeptide chains of said binding protein form four functional antigen binding sites, wherein at least one heavy chain variable domain an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7 and 9, and at least one light chain variable domain comprises an amino acid sequence having at least 90% identity with a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10.

40. The binding protein according to claim 39, wherein the binding protein is capable of binding HCV core protein.

41. The binding protein according to claim 39, wherein the binding protein is capable of modulating a biological function of HCV core protein.

42. The binding protein according to claim 39, wherein the binding protein is capable of neutralizing HCV core protein.

43. The binding protein according to claim 39, comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOS: 48-79.

44. The binding protein according to claim 39, comprising at least one variable heavy chain domain and one variable light chain domain amino acid sequence selected from the group consisting of SEQ ID NOS: 48-79.

45. A pharmaceutical or diagnostic composition comprising: a binding protein according to any one of claims 39-44, and a pharmaceutically acceptable carrier, diluent or excipient.

46. The pharmaceutical or diagnostic composition according to claim 45, further comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain.

47. A method of reducing or eliminating human hepatitis C virus activity in a human subject, the method comprising: administering to a subject in need thereof an effective amount of a binding protein according to any one of claims 39-44.

48. The method of claim 47, further comprising administering to the subject an effective amount of a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain.

49. A method of detecting the presence of human hepatitis C virus in a sample, the method comprising: contacting the sample with a binding protein according to any one of claims 39-44.

50. The method according to claim 49, further comprising contacting the sample with a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain.

51. An immunoassay method for determining the presence or amount of human hepatitis C virus in a sample, comprising: providing a reagent comprising a binding protein according to any one of claims 39-44; combining the binding protein reagent with the sample for a time sufficient for the binding protein reagent to bind to any human hepatitis C virus in the sample; and determining the presence or amount of human hepatitis C virus present in the sample based on specific binding of the binding protein reagent to the human hepatitis C virus core protein.

52. The immunoassay method according to claim 51, wherein the binding protein reagent is immobilized or is capable of being immobilized on a solid support.

53. The immunoassay method according to claim 51, wherein the binding protein reagent is labeled with a detectable label.

54. The immunoassay method according to claim 51, further comprising providing a reagent comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, and combining the monoclonal antibody reagent with the sample for a time sufficient for the monoclonal antibody reagent to bind to any human hepatitis C virus in the sample.

55. The immunoassay method according to claim 54, wherein the monoclonal antibody reagent is immobilized or is capable of being immobilized on a solid support.

56. An immunoassay device for detecting the presence or absence of human hepatitis C virus in a sample, the device comprising: a binding protein according to any one of claims 39-44 immobilized on a solid support.

57. The immunoassay device according to claim 56, further comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain, immobilized on the solid support.

58. A kit for detecting the presence or absence of human hepatitis C virus in a sample, the kit comprising: a binding protein according to any one of claims 39-44; and instructions for determining whether the sample contains human hepatitis C virus.

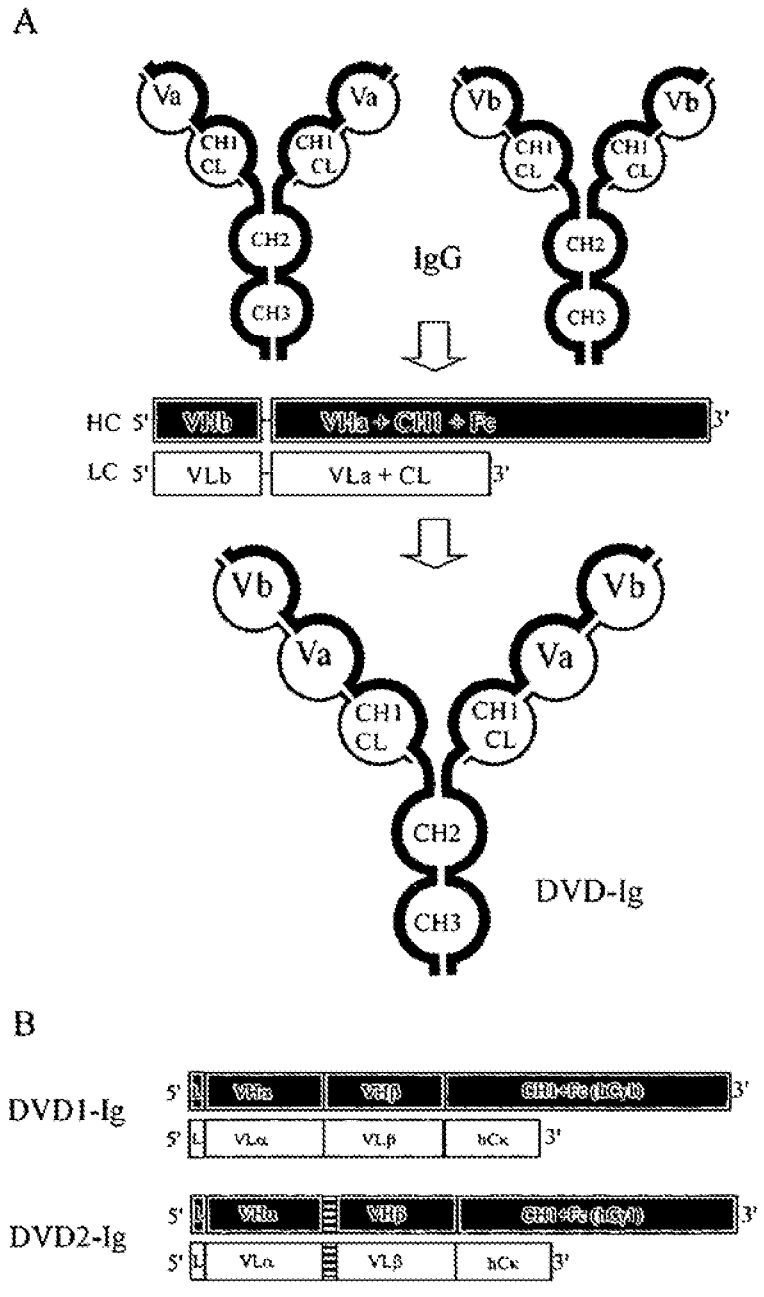
59. The kit according to claim 58, wherein the binding protein is immobilized on a solid support.

60. The kit according to claim 58, further comprising a monoclonal antibody that specifically binds human hepatitis C virus core protein and which has a heavy chain and a light chain.

61. The kit according to claim 60, wherein the monoclonal antibody is immobilized on the solid support.

62. The kit according to claim 58, further comprising a detectable label.

Figure 1


A
Va
Vb
CH1
CL
CH2
CH3
IgG
HC 5'
VHb
VHa + CH1 + Fc
3'
LC 5'
VLb
VLa + CL
3'
DVD-Ig
B
DVD1-Ig
DVD2-Ig
5'
3'
VLα
VLβ
hCκ