



(86) Date de dépôt PCT/PCT Filing Date: 2005/03/10
(87) Date publication PCT/PCT Publication Date: 2006/02/02
(85) Entrée phase nationale/National Entry: 2006/11/25
(86) N° demande PCT/PCT Application No.: US 2005/007859
(87) N° publication PCT/PCT Publication No.: 2006/011919
(30) Priorité/Priority: 2004/06/30 (US60/584,715)

(51) Cl.Int./Int.Cl. *C07K 14/155* (2006.01),
G01N 33/569 (2006.01)
(71) Demandeur/Applicant:
IDEXX LABORATORIES, INC., US
(72) Inventeurs/Inventors:
GROAT, RANDALL GENE, US;
TONELLI, QUENTIN JOSEPH, US
(74) Agent: SMART & BIGGAR

(54) Titre : PROCEDE ET APPAREIL SERVANT A DETECTER LE VIRUS DE L'IMMUNODEFICIENCE FELINE
(54) Title: METHOD AND DEVICE FOR DETECTING FELINE IMMUNODEFICIENCY VIRUS

(57) **Abrégé/Abstract:**

A method and device for determining a feline immunodeficiency virus infection or vaccination in an animal. The method includes contacting a biological sample from a felid with various FIV polypeptides and determining the binding of antibodies in the sample to the polypeptides. The determination of whether an animal is infected with FIV or has been vaccinated against FIV can be determined by measuring the animal's immune response to an FIV env polypeptide. A device for detecting FIV antibodies is provided.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 February 2006 (02.02.2006)

PCT

(10) International Publication Number
WO 2006/011919 A1

(51) International Patent Classification⁷: **C07K 14/155**,
G01N 33/569

(21) International Application Number:
PCT/US2005/007859

(22) International Filing Date: 10 March 2005 (10.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/584,715 30 June 2004 (30.06.2004) US

(71) Applicant (for all designated States except US): **IDEXX LABORATORIES, INC.** [US/US]; One IDEXX Drive, Westbrook, ME 04092 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GROAT, Randall, Gene** [US/US]; 19 Holbrook Street No. 11, Freeport, ME 04032 (US). **TONELLI, Quentin, Joseph** [US/US]; 37 Wellington Road, Portland, ME 04103 (US).

(74) Agent: **SWARTZ, Jennifer, M.**; McDonnell Boehnen Hulbert & Berghoff LLP, 300 South Wacker Drive, Suite 3100, Chicago, IL 60606 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

METHOD AND DEVICE FOR DETECTING FELINE IMMUNODEFICIENCY VIRUS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/584,715, filed June 30, 2004.

FIELD OF THE INVENTION

[0002] The invention is related to the detection of antibodies directed to Feline Immunodeficiency Virus.

BACKGROUND OF THE INVENTION

[0003] Feline immunodeficiency virus (FIV), formerly called feline T-lymphotrophic lentivirus, was first isolated in 1986 from a large multiple cat household in Petaluma, Calif. (Pederson et al., Science (1987) 235:790). FIV infects cats to produce an AIDS-like syndrome. Although FIV is morphologically and pathologically similar to the human immunodeficiency virus (HIV), it has been shown to be antigenically distinct from HIV. Like HIV, once a cat becomes infected with FIV, the disease progresses from a primary infection (viraemia, fever, general lymphadenitis) to a lengthy asymptomatic phase, followed by severe impairment in immune function caused by a reduction in CD4 lymphocytes, and resulting in heightened susceptibility to secondary infections and ultimately death.

[0004] FIV has been classified as a member of the subfamily Lentiviridae in the family Retroviridae, the family that includes human and simian immunodeficiency viruses, equine infectious anaemia, maedi visna of sheep and caprine arthritis encephalitis viruses (CAEV). The genome of FIV is organized like other lentiviruses with three long open reading frames corresponding to *gag*, *pol* and *env* (Talbot et al., Proc. Natl. Acad. Sci. (1989) 86:5743; Olmsted et al., Proc. Natl. Acad. Sci. (1989)

86:2448). The *gag* gene codes for the major structural components of the virus, the *env* gene codes for the envelope glycoprotein, and the *pol* gene codes for the polymerase protein.

[0005] The *gag* gene is expressed as a 55 kD polyprotein that is processed into three subunits: a p15 matrix protein, a p24 capsid protein, and a p10 nucleocapsid protein. The *pol* gene encodes three proteins: the protease, reverse transcriptase and a p14.6 protein of unknown function. Autoprocessing by the protease portion of the gene gives rise to all three proteins of the *pol* region. Additionally, the protease is responsible for the processing of the *gag* precursor. The *pol* gene is expressed as a *gag-pol* fusion protein. The envelope gene is expressed as a 160 kD glycoprotein, gp160. The antigenicity of the FIV core proteins is similar to other lentiviruses.

[0006] Several independent viral isolates have been prepared across the world, and a certain number of studies have been carried out in order to demonstrate the structure of the isolated strains: the American strain Petaluma, Talbott *et al.* Natl. Acad. Sci. USA, 1989, 86, 5743-5747; Philipps *et al.*, J. Virol., 1990, 64, 10, 4605-4613, the Japanese strains (the TM1 and TM2 strains), Miyazawa *et al.*, Arch. Virol., 1989, 108, 59-68, and the Swiss isolates (FIVZ1 and FIVZ2), Morikawa *et al.*, Virus Research, 1991, 21, 53-63.

[0007] The nucleotide sequences of three proviral clones derived from American FIV isolates (Petaluma strain) have been described (clones FIV34TF10, FIV14 and isolate PPR) (Olmsted, *et al.* 1989; Philipps *et al.*, 1990; Talbott *et al.*, 1989) and compared with two Swiss isolates (Morikawa *et al.* 1991). This comparison led Morikawa *et al.* to specify the presence of certain conserved regions and certain

variable regions within the *env* gene of FIV. French strains have also been isolated (strains Wo and Me)(Moraillon *et al.*, 1992, Vet. Mic., 31, 41-45).

[0008] The virus replicates optimally in blood mononuclear cells and has a tropism for T-lymphocytes, peritoneal macrophage, brain macrophage and astrocytes. In common with other retroviruses, the genetic material of FIV is composed of RNA and the production of a DNA copy of the viral RNA is an essential step in the replication of FIV in the host. This step requires the enzyme reverse transcriptase that is carried into the host by the invading virus. The DNA version of the viral genome is inserted into the genetic material of infected host cells in which it continues to reside as a provirus. This provirus is replicated every time the cell divides and can code for the production of new virus particles. Cells infected with FIV remain infected for the duration of their lifespan.

[0009] The virus appears to be spread naturally by horizontal transmission, predominantly by bite wounds from an infected cat as these animals shed appreciable amounts of virus in saliva (Yamamoto *et al.*, Am. J. Vet. Res. 1988, 8:1246). Vertical transmission has been reported, but is rare.

[0010] Current diagnostic screening tests for FIV infection detect serum antibody (Ab) to FIV. Virus detection kits are also available but not as prevalent. A number of diagnostic tests are available to determine the presence of FIV antibody in infected animals. For example, PetChek® FIV Ab test kit and the SNAP® Combo FeLV Ag/FIV Ab test kit (IDEXX Laboratories, Westbrook, Maine) are immunoassay based diagnostic tests for FIV infection.

[0011] Detecting FIV infection is becoming increasingly important as studies reveal FIV infection is widespread worldwide. As vaccines have been developed in

attempt to combat the disease, it is even more important to be able to detect the effectiveness of a vaccine and to discriminate between vaccinated cats versus naturally infected cats.

SUMMARY OF THE INVENTION

[0012] In one aspect, the invention is directed to novel FIV polypeptides. In another aspect, the invention provides a method for determining whether a felid has been vaccinated against FIV or is naturally infected with FIV by determining the felid's immune response to an FIV polypeptide, such as an FIV *env* polypeptide.

[0013] In another aspect, the invention is directed to a method of distinguishing among animals that have been naturally infected with FIV versus animals that have not been infected or have been vaccinated against an FIV infection. The method includes contacting a biological sample from an animal with a polypeptide that does not substantially bind to an FIV antibody that is a significant component of the animal's immune response to an FIV vaccine. FIV antibodies in the sample that substantially bind to the polypeptide are detected. It is determined that the animal is naturally infected by correlating a positive result in the detecting step to a natural infection and it is determined that the animal has been vaccinated or not infected by correlating a negative result to a vaccination or no infection. The polypeptide may be derived from FIV *env*.

[0014] In a further aspect, the invention is directed to a method of determining whether a cat has been vaccinated against FIV or is naturally infected with FIV. The method includes (a) detecting, before a period a time following vaccination sufficient for certain FIV antigen-specific antibodies raised in response to the vaccine to be not detected, whether the cat has antibodies against an FIV peptide; (b) detecting, after a

period of time following vaccination sufficient for certain FIV antigen-specific antibodies raised in response to the vaccine to be not detected, whether the cat has antibodies against an FIV polypeptide; (c) determining that the animal has been vaccinated by detecting antibodies in step a but not in step b; and (d) determining that the animal is naturally infected by detecting antibodies in steps a and b.

[0015] The invention also provides for a method of determining whether a cat has not been infected by FIV or has been vaccinated against FIV. The method includes analyzing a biological sample from the cat to detect antibodies against a polypeptide derived from FIV, and determining that the animal has not been infected or has been or vaccinated by not detecting such antibodies.

[0016] In yet another aspect, the invention provides a method of determining whether or not a cat has been vaccinated for FIV or naturally infected with FIV. The method includes providing a test device comprising a polypeptide, obtaining a blood sample from a cat, running the blood sample on the test device, and reading the test device. A positive result indicates the cat has been naturally infected with FIV or vaccinated against FIV and a negative result indicates the cat has not been naturally infected with FIV and not vaccinated against FIV.

[0017] Still further, the invention is directed to a diagnostic device having a dry porous carrier, a first detection reagent immobilized on the porous carrier where the first detection reagent includes a protein that captures FIV antibodies generated by a host in response to either a natural FIV infection or an FIV vaccination, and a second detection reagent immobilized on the porous carrier wherein the second detection reagent includes a protein that captures FIV antibodies generated by a host in response to a natural FIV infection but does not substantially capture antibodies

generated by the host in response to an FIV vaccination. The first detection reagent may be FIV p15 or p24 antigen, and the second detection reagent may be an FIV *env* protein.

DETAILED DESCRIPTION

[0018] Before describing the present invention in detail, a number of terms will be defined. As used herein, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0019] As used herein, the term "polypeptide" refers to a compound of a single chain or a complex of two or more chains of amino acid residues linked by peptide bonds. The chain(s) may be of any length. A protein is a polypeptide and the terms are used synonymously. Also included within the scope of the invention are functionally equivalent variants and fragments of FIV polypeptides. The polypeptide is capable of binding one or more antibodies specific for the polypeptide.

[0020] Polypeptides derived from FIV include any region of the of the FIV proteome including for example, portions of the *gag* and *env* regions and mimitopes thereof. U.S. Patent Nos. 5,648,209, 5,591,572, and 6,458,528, which are incorporated by reference herein in their entirety, describe FIV polypeptides derived from the FIV *env* and *gag* proteins. These peptides, and others like them, from the *env* and *gag* proteins, are suitable for use in the methods of the present invention. An example of a suitable *env* polypeptide includes the following:

CELGCNQNQFFCK [SEQ ID NO:1]

[0021] SEQ ID NO:1 is the native FIV *env* sequence, amino acids 696 – 707, shown here with a non-native N-terminal cysteine residue.

[0022] “Binding specificity” or “specific binding” refers to the substantial recognition of a first molecule for a second molecule, for example a polypeptide and a polyclonal or monoclonal antibody, or an antibody fragment (*e.g.* a Fv, single chain Fv, Fab’, or F(ab’)2 fragment) specific for the polypeptide.

[0023] “Substantial binding” or “substantially bind” refer to an amount of specific binding or recognizing between molecules in an assay mixture under particular assay conditions. In its broadest aspect, substantial binding relates to the difference between a first molecule's incapability of binding or recognizing a second molecule, and the first molecules capability of binding or recognizing a third molecule, such that the difference is sufficient to allow a meaningful assay to be conducted distinguishing specific binding under a particular set of assay conditions, which includes the relative concentrations of the molecules, and the time and temperature of an incubation. In another aspect, one molecule is substantially incapable of binding or recognizing another molecule in a cross-reactivity sense where the first molecule exhibits a reactivity for a second molecule that is less than 25%, preferably less than 10%, more preferably less than 5% of the reactivity exhibited toward a third molecule under a particular set of assay conditions, which includes the relative concentration and incubation of the molecules. Specific binding can be tested using a number of widely known methods, *e.g.* an immunohistochemical assay, an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), or a western blot assay.

[0024] Animals infected with FIV are felids, which is to be understood to include all members of the order Felidae, including domestic cats, lions, tigers, jaguars, leopards, puma, ocelots, etc. As used herein, the terms “felid,” “cat” or “animal” is a reference to all felids.

[0025] A “biological sample” refers to a sample from an animal subject including saliva, whole blood, serum, plasma or other sample known to contain FIV antibodies.

[0026] An “antibody that is a significant component of an animal’s immune response to a FIV vaccine” refers to an antibody that is elicited as the result of a vaccination with a FIV vaccine. These antibodies may be identical to or similar to antibodies elicited as the result of a natural FIV infection. A successful vaccination produces a measurable level of the antibody that is a significant component of an animal’s immune response to the FIV vaccine.

[0027] Vaccines for FIV are described, for example, in U.S. Patents 6,667,295, 5,833,993, 6,447,993, 6,254,872 and 6,544,528, and published U.S. Patent Application 20040096460, each of which is incorporated herein by reference in their entirety. U.S. patents 6,447,993 and 6,254,872 describe vaccines that are prepared from cell free-viral isolates of different FIV subtypes or a combination of cell lines each infected with different prototype FIV virus from a different subtype. U.S. Patent 5,833,933 describes vaccines containing DNA sequences encoding FIV *gag* protein and FIV *env* protein. These vaccines include an expression system for expressing the sequences. One available vaccine is FEL-O-VAX® FIV (Fort Dodge Animal Health, Overland Park, Kansas).

[0028] Biological samples from animals that have been vaccinated against an FIV infection have the potential for producing a positive result in a test for an FIV infection due to the presence of antibodies produced by the animal in response to the vaccine. In one aspect, the invention provides for a method of distinguishing between animals that have been naturally infected with FIV and animals that have not been infected or have been vaccinated against an FIV infection. The method includes

contacting a biological sample from the animal with a polypeptide derived from an FIV that does not substantially bind to an antibody that is a significant component of the animal's antibody response to an FIV vaccine.

[0029] In another aspect, the invention includes a method of determining whether a cat has not been infected by FIV and has not been vaccinated against FIV. A biological sample from a cat is analyzed to detect antibodies against a polypeptide, derived from FIV *env* and/or *gag*. It is then determined that the animal has not been infected and has not been vaccinated by determining the absence of such antibodies.

[0030] In some instances, during an initial phase following a vaccination, an animal may temporarily (transiently) produce lower levels of certain antibodies to specific FIV polypeptides that are elements of a vaccine, as compared to those produced in response to a natural infection. These antibody levels taper off after a period of time to the point that antibody to these polypeptides is not detected after the initial phase. Generally, this amount of time is about ten to twelve weeks, but will vary between species and individual subject animals. Transient antibodies are not a significant component of the animal's immune response to the vaccine.

[0031] For example, the development of FIV antibodies in an animal against a vaccine is dependent upon the vaccine. It has been found that animals test seropositive for FIV antibodies against p24 (*gag*) about two to four weeks after vaccination with the FEL-O-VAX® vaccine. However, animals so vaccinated do not generate persistent levels of antibodies against one or more regions of the *env* protein, even though this protein was included as an element of the vaccine. In contrast,

naturally infected animals typically generate persistent levels of antibodies to both FIV *gag* and *env* proteins.

[0032] The differences in the immune response between animals that are vaccinated and animals that are naturally infected provide a means for determining whether an animal has been vaccinated or is naturally infected. Using the method of the invention, animals that have been naturally infected with FIV can be distinguished from animals that have not been infected or have been vaccinated against an FIV infection. Accordingly, the detection of the substantial binding between a polypeptide derived from FIV and an antibody that is not a significant component of an animal's immune response to a vaccine can indicate a natural infection. The relative absence of such binding can indicate vaccination or no infection. In addition, a second, separate antibody capture reagent can be included in the test that substantially binds to antibodies produced in response to vaccination and/or natural infection, such as p15 or p24 proteins. As such, various combinations of separate capture reagents can lead to a determination of the vaccination and/or infection status of the test subject.

[0033] For example, FIV *gag* proteins p15 and p24 may be immunogenic components of a killed whole virus FIV vaccine. It is expected that these components elicit a persistent antibody response when administered to an animal. On the other hand, some vaccines may not include immunologically significant quantities of FIV *env* protein or, this protein has been altered in the process of virus inactivation, or presentation of this protein by vaccination differs from that for natural infection to a point where antibodies produced thereto, if any, are detected for a period of time less than antibodies to p15 and p24. Thus, while during the initial phase following

vaccination, an animal may transiently produce low levels of such antibodies that bind to *env* proteins, any such antibody production declines over a period of time and is not detected after about 12 weeks. In this example, the transiently produced antibodies are not a significant component of the animal's immune response to the vaccine after a period of time.

[0034] Given that the production of detectable antibodies that are directed toward specific FIV *env* polypeptides usually drops off after about 12 weeks from completion of vaccination, in one aspect of the invention the biological sample is obtained from the animal that has not received an FIV vaccine within about the prior 12 weeks. If the vaccination status is unknown and the test indicates infection (based on a reaction with the antibody capture protein), a retest after an additional 12 weeks can be recommended.

[0035] Differences in the immune response between vaccinated animals and naturally infected animals, such as specific antibody levels and/or kinetic parameters for antibody-antigen binding reactions (e.g., affinities, avidities), should be considered in the design of an assay for distinguishing between vaccinated and infected animals. Differences in immune response can be significant such that even after the initial phase following a vaccination, an animal may persistently produce lower levels of antibodies to specific FIV polypeptides, and/or antibodies with different binding properties as compared to those antibodies produced in response to a natural infection.

[0036] The method of the invention can be optimized in many ways and one of skill in the art could simultaneously adjust the sample dilutions, reagent concentrations, incubation temperatures and times used in the method to accomplish a differential detection of serum having antibodies to an FIV infection or vaccination.

For instance, at optimized sample dilution and other conditions for an immunoassay for antibodies to specific polypeptides, samples from vaccinated animals may, for one specific FIV polypeptide, give a negative assay result and samples from infected animals will give a positive assay result. For a second FIV polypeptide, both samples may give a positive result.

[0037] In one aspect of the invention, the proteins are immobilized on a suitable solid support. The biological sample is brought into contact with the protein, to which the anti-FIV antibodies bind, if such antibodies are present in the sample. The binding may be detected by any suitable means, e.g., enzymes, radionuclides, particulates or fluorescent labels. In a suitable embodiment, the detection reagent can be associated with a protein that is the same or similar to that which is used to capture anti-FIV antibodies (if present).

[0038] In another aspect, the method is directed to a test device to determine whether a cat has been vaccinated for FIV or naturally infected with FIV. A method of using the test device includes providing a test device having an FIV *env* protein and a separate FIV *gag* protein. The device can be used to test a biological sample from a cat by contacting the device with the biological sample. Upon reading the device, the detection of the binding of an antibody to the *gag* protein (a positive result on the *gag protein*) indicates the cat has been naturally infected with FIV or vaccinated against FIV. A concurrent positive result on the *env* protein indicates natural infection (or, perhaps a transient post vaccination response), while a concurrent negative result on the *env* protein indicates vaccination. The following table summarizes the above:

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	<u><i>gag</i> protein</u>	<u><i>env</i> protein</u>
No vaccination or infection	-	-
Vaccination	+	-
Potential Recent Vaccination	+	+
Infection	+	+
Infection and Vaccination	+	+

[0039] The polypeptides used in the invention contain at least six amino acids, usually at least nine amino acids, and more usually twelve or more amino acids found within one of the natural FIV proteins and mimitopes and functionally equivalent variants thereof.

[0040] “Functional equivalent” or “Functionally equivalent” refers to polypeptides related to or derived from the native FIV envelope (*env*) and viral core (*gag*) polypeptide sequences where the amino acid sequence has been modified by a single or multiple amino acid substitution, insertion, deletion, and also sequences where the amino acids have been chemically modified, such as amino acid analogs, but which nonetheless retain substantially equivalent function. Functionally-equivalent variants may occur as natural biological variations or may be prepared using known techniques such as chemical synthesis, site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of amino acids. Thus, modification of the amino-acid sequence to obtain variant sequences may occur so long as the function of the polypeptide is not affected.

[0041] FIV functionally-equivalent variants within the scope of the invention may comprise conservatively substituted sequences, meaning that one or more amino acid residues of the FIV polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the FIV polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical properties, such as charge density, size, configuration, or hydrophilicity/hydrophobicity. For purposes of example only, such substitutions could include substituting one aliphatic residue (Ile, Val, Leu, or Ala) for another, or substitution of basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Conservative variants can generally be identified by modifying a polypeptide sequence of the invention and evaluating the antigenic activity of the modified polypeptide using, for example, an immunohistochemical assay, an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), or a western blot assay. Further information regarding the making of phenotypically silent amino acid exchanges may be found in Bowie *et al.*, *Science* 247:1306-1310 (1990).

[0042] Examples of functional equivalents of SEQ ID NO: 1 are shown here with a description of the various modifications to the peptides.

SEQ ID NO:	Sequence	Description of Amino Acid Changes
2	CELGSNQNQFFSK	Native FIV env sequence, amino acids 696 – 707, N-terminal C addition, C to S substitutions
3	KVEAMEKFLYTAFAMQELGCN QNQFFCKIPLELWTR	Native FIV env sequence, amino acids 680 – 715
4	TAFAMQELGSNQNQFFSK	Native FIV env sequence, amino acids 690 – 707, C to S substitutions
5	YTAFAMQE <u>W</u> GCNQNQFFC <u>A</u>	Native FIV env sequence, amino acids 689 – 707, L to W substitution, K to A substitution
6	TAFAMQELGCNQ <u>Q</u> QFFC <u>A</u>	Native FIV env sequence, amino acids 690 – 707, N to Q, K to A substitutions

7	YTAFAMQEIGCNQNQFFCA	Native FIV env sequence, amino acids 689 – 707, L to I, K to A substitutions
8	CEGSNQNQFFSK	Native FIV env sequence, amino acids 696 – 707, N-terminal C addition; L deletion, C to S substitutions

[0043] Additional variants are also contemplated within the scope of the invention, and such variants include amino and/or carboxyl terminal fusions, for example achieved by addition of amino acid sequences of any number of residues, as well as intrasequence insertion of one or more amino acids. For example, amino acid sequences added may be those derived from the whole or parts of other polypeptides or proteins, or may be those provided in the corresponding positions in the FIV envelope or viral protein. Longer peptides may comprise multiple copies of one or more of the polypeptide sequences. Moreover, multiple copies of the polypeptides may be coupled to a polyamino acid backbone, such as a polylysine backbone to form multiple antigen peptides (MAPs).

[0044] Deletional amino acid sequence variants are those in which one or more amino acid residues are removed from the sequence. Insertional variants exist when one or more amino acids are integrated into a predetermined site in the protein, although random insertion is an option with suitable screening of the resulting product. In all cases, these and other FIV variants used retain substantially the same antigenicity of the FIV polypeptides. Other variants are also contemplated, including those where the amino acid substitutions are made in the area outside the antibody recognition regions of the protein. Fusion proteins comprising two or more polypeptide sequences of FIV are also within the scope of the invention provided the sequences provide the appropriate antigenicity. Such polypeptides will generally correspond to at least one epitope or mimitope that is characteristic of FIV. By characteristic, it is meant that the epitope or mimitope will allow immunologic

detection of antibody directed to FIV in a physiological sample with reasonable assurance. Usually, it will be desirable that the epitope or mimitope, variant or fusion protein be immunologically distinct from (i.e., not cross-reactive with antibodies which recognize) viruses other than FIV.

[0045] An antigenically active variant differs by about, for example, 1, 2, 3, 4, 5, or 6 amino acid residues from SEQ ID NO: 1, such as those shown in SEQ ID NOS: 2 – 8, or a fragment thereof. Where this comparison requires alignment the sequences are aligned for maximum homology. Deletions, insertions, substitutions, repeats, inversions or mismatches are considered differences. The differences are, preferably, differences or changes at a non-essential residue or a conservative substitution. The site of variation can occur anywhere in the polypeptide, as long as the resulting variant polypeptide is antigenically substantially similar to SEQ ID NO: 1, such as, for example, the variations shown in SEQ ID NOS: 2-8 (see Tables 2 and 3). Exemplary functionally-equivalent variants include those displaying 50% or more amino acid homology. Preferably, such homology is 60%, 70%, or greater than 80%. However, such variants may display a smaller percentage of homology overall and still fall within the scope of the invention where they have conserved regions of homology.

[0046] In some cases, one or more cysteine residues may be added to the termini of the polypeptides in order to facilitate specific carrier linkage or to permit disulphide bonding to mimic antigenic loops and thus increase the antigenicity. Moreover, a fatty acid or hydrophobic tail may be added to the peptides to facilitate incorporation into delivery vehicles and to increase antigenicity.

[0047] The FIV polypeptides used as detection reagents may be natural, i.e., including the entire FIV protein or fragments thereof isolated from a natural source, or may be synthetic. The natural proteins may be isolated from the whole FIV virus by conventional techniques, such as affinity chromatography. Polyclonal or monoclonal antibodies may be used to prepare a suitable affinity column by well-known techniques.

[0048] Proteins that are immunologically cross-reactive with a natural FIV protein can be chemically synthesized. For example, polypeptides having fewer than about 100 amino acids, more usually fewer than about 80 amino acids, and typically fewer than about 50 amino acids, may be synthesized by the well-known Merrifield solid-phase synthesis method where amino acids are sequentially added to a growing chain. (Merrifield, 1963, J. Am. Chem. Soc., 85:2149-2156). Recombinant proteins can also be used. These proteins may be produced by expression in cultured cells of recombinant DNA molecules encoding a desired portion of the FIV genome. The portion of the FIV genome may itself be natural or synthetic, with natural genes obtainable from the isolated virus by conventional techniques. Of course, the genome of FIV is RNA, and it will be necessary to transcribe the natural RNA into DNA by conventional techniques employing reverse transcriptase. Polynucleotides may also be synthesized by well-known techniques. For example, short single-stranded DNA fragments may be prepared by the phosphoramidite method described by Beaucage and Carruthers, 1981, Tett. Letters 22:1859-1862. Double-stranded fragments may then be obtained either by synthesizing the complementary strand and then annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

[0049] The natural or synthetic DNA fragments coding for the desired FIV protein or fragment may be incorporated in a DNA construct capable of introduction to and expression in *in vitro* cell culture. Usually, the DNA constructs will be suitable for replication in a unicellular host, such as yeast or bacteria. They may also be intended for introduction and integration within the genome of cultured mammalian or other eukaryotic cells. DNA constructs prepared for introduction into bacteria or yeast will include a replication system recognized by the host, the FIV DNA fragment encoding the desired polypeptide product, transcriptional and translational initiation regulatory sequences joined to the 5'-end of the FIV DNA termination regulatory sequences joined to the 3'-end of the fragment. The transcriptional regulatory sequences will include a heterologous promoter that is recognized by the host. Conveniently, a variety of suitable expression vectors are commercially available for a number of hosts.

[0050] To be useful in the detection methods of the present invention, the polypeptides are obtained in a substantially pure form, that is, typically from about 50% w/w or more purity, substantially free of interfering proteins and contaminants. Preferably, the FIV polypeptides are isolated or synthesized in a purity of at least 80% w/w, and more preferably, in at least about 95% w/w purity. Using conventional protein purification techniques, homogeneous polypeptide compositions of at least about 99% w/w purity can be obtained. For example, the proteins may be purified by use of the antibodies described hereinafter using the immunoabsorbant affinity columns described hereinabove.

[0051] The method of the invention may be accomplished using immunoassay techniques well known to those of skill in the art, including, but not limited to, using

microplates and lateral flow devices. In one embodiment, an FIV protein is immobilized on a solid support at a distinct location. Detection of protein-antibody complexes on the solid support can be by any means known in the art. For example, U.S. Patent No. 5,726,010, which is incorporated herein by reference in its entirety, describes an example of a lateral flow device, the SNAP® immunoassay device (IDEXX Laboratories), useful in the present invention. Colloidal particle based tests can also be used, such as the commercially available WITNESS® FIV diagnostic test (Synbiotics Corporation, Lyon, France).

[0052] Immobilization of one or more analyte capture reagents, *e.g.*, FIV proteins, onto a device or solid support is performed so that an analyte capture reagent will not be washed away by the sample, diluent and/or wash procedures. One or more analyte capture reagents can be attached to a surface by physical adsorption (*i.e.*, without the use of chemical linkers) or by chemical binding (*i.e.*, with the use of chemical linkers). Chemical binding can generate stronger attachment of specific binding substances on a surface and provide defined orientation and conformation of the surface-bound molecules.

[0053] Another embodiment of the invention provides a device that is suitable for a lateral flow assay. For example, a test sample is added to a flow matrix at a first region (a sample application zone). The test sample is carried in a fluid flow path by capillary action to a second region of the flow matrix where a label capable of binding and forming a first complex with an analyte in the test sample. The first complex is carried to a third region of the flow matrix where an FIV protein is immobilized at a distinct location. A second complex is formed between an immobilized protein and the first complex including the antibody from the sample. For example, a first

complex comprising a gold sol particle and an FIV protein bound to an FIV antibody will specifically bind and form a second complex with a second immobilized FIV protein or with a second antibody directed to feline antibodies. The label that is part of the second complex can be directly visualized.

[0054] In another aspect, the invention includes one or more labeled specific binding reagents that can be mixed with a test sample prior to application to a device for of the invention. In this case it is not necessary to have labeled specific binding reagents deposited and dried on a specific binding reagent pad in the device. A labeled specific binding reagent, whether added to a test sample or pre-deposited on the device, can be for example, a labeled FIV protein that specifically binds an antibody for FIV.

[0055] Any or all of the above embodiments can be provided as a kit. In one particular example, such a kit would include a device complete with specific binding reagents (*e.g.*, a non-immobilized labeled specific binding reagent and an immobilized analyte capture reagent) and wash reagent, as well as detector reagent and positive and negative control reagents, if desired or appropriate. In addition, other additives can be included, such as stabilizers, buffers, and the like. The relative amounts of the various reagents can be varied, to provide for concentrations in solution of the reagents that substantially optimize the sensitivity of the assay. Particularly, the reagents can be provided as dry powders, usually lyophilized, which on dissolution will provide for a reagent solution having the appropriate concentrations for combining with a sample.

[0056] An FIV protein can be an immobilized analyte capture reagent in a reaction zone (solid phase). A second analyte capture reagent, *i.e.* a second FIV

protein, that has been conjugated to a label, can either be added to the sample before the sample is added to the device, or the second analyte capture reagent can be incorporated into the device. For example the labeled specific binding reagent can be deposited and dried on a fluid flow path that provides fluid communication between the sample application zone and the solid phase. Contact of the labeled specific binding reagent with the fluid sample results in dissolution of the labeled specific binding reagent.

[0057] The device may also include a liquid reagent that transports unbound material (*e.g.*, unreacted fluid sample and unbound specific binding reagents) away from the reaction zone (solid phase). A liquid reagent can be a wash reagent and serve only to remove unbound material from the reaction zone, or it can include a detector reagent and serve to both remove unbound material and facilitate analyte detection. For example, in the case of a specific binding reagent conjugated to an enzyme, the detector reagent includes a substrate that produces a detectable signal upon reaction with the enzyme-antibody conjugate at the reactive zone. In the case of a labeled specific binding reagent conjugated to a radioactive, fluorescent, or light-absorbing molecule, the detector reagent acts merely as a wash solution facilitating detection of complex formation at the reactive zone by washing away unbound labeled reagent.

[0058] Two or more liquid reagents can be present in a device, for example, a device can comprise a liquid reagent that acts as a wash reagent and a liquid reagent that acts as a detector reagent and facilitates analyte detection.

[0059] A liquid reagent can further include a limited quantity of an "inhibitor", *i.e.*, a substance that blocks the development of the detectable end product. A limited

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quantity is an amount of inhibitor sufficient to block end product development until most or all excess, unbound material is transported away from the second region, at which time detectable end product is produced.

[0060] The following are provided for exemplification purposes only and are not intended to limit the scope of the invention described in broad terms above. All references cited in this disclosure are incorporated herein by reference.

Examples

Example 1

[0061] Eight cats testing negative for FIV with the SNAP® FeLV Ag/FIV Ab test kits were vaccinated with Fel-O-Vax® FIV vaccine, Fort Dodge Animal Health, Fort Dodge Iowa. This vaccine is produced from multiple strains of the whole killed FIV virus. The cats were vaccinated following the manufacturer's directions at day 0, 14, and 28. Two cats testing negative for FIV were not vaccinated and were included as controls for this study.

[0062] Blood samples were obtained from each of the ten cats in the vaccination study at day zero and every seven days for 12 weeks and stored frozen until testing. In addition, blood samples from FIV negative cats and cats naturally infected with FIV, confirmed FIV Ab negative or positive by a western immunoblot confirmatory test, were also tested.

[0063] Sample testing was accomplished using a SNAP® ELISA format. SNAP® device technology was used to provide a solid phase with reversible, chromatographic flow of sample, and automatic, sequential flow of wash and enzyme substrate solutions as described in U.S. Patent 5,726,010.

[0064] For the SNAP® device, FIV *gag* p24 (recombinant) and an FIV *env* 696-707 with additional N-terminal cysteine -- CELGCNQNQFFCK [SEQ ID NO:1] -- proteins were deposited to form a single antibody capture spot on the solid phase. A negative control reagent was deposited to form a negative control spot and a positive control reagent was deposited to form a positive control spot on the solid phase of the SNAP® device. The *gag* or *env* proteins were chemically conjugated to the enzyme horseradish peroxidase and provided in a solution consisting of a buffer, detergent, and animal serum components.

[0065] Serum samples were combined with either *gag* or *env* protein-enzyme conjugate solution, and applied to the SNAP® device. Following a short incubation period, the device was activated. Color development on the positive control spot indicated the test was valid. Color development on the sample spot greater than color development on the negative control spot indicated the presence of FIV antibody in the sample and was scored as a positive test result. Test results were determined visually and are shown in Table 1.

Table 1

Animal ID	Status	Day	<i>gag</i> Ab test result (visual)	<i>env</i> Ab test result (visual)
NV1	not vaccinated, not infected	0	NEG	NEG
NV1	not vaccinated, not infected	7	NEG	NEG
NV1	not vaccinated, not infected	14	NEG	NEG
NV1	not vaccinated, not infected	21	NEG	NEG
NV1	not vaccinated, not infected	28	NEG	NEG
NV1	not vaccinated, not infected	35	NEG	NEG
NV1	not vaccinated, not infected	42	NEG	NEG
NV1	not vaccinated, not infected	49	NEG	NEG
NV1	not vaccinated, not infected	56	NEG	NEG
NV1	not vaccinated, not infected	63	NEG	NEG
NV1	not vaccinated, not infected	70	NEG	NEG
NV1	not vaccinated, not infected	77	NEG	NEG
NV1	not vaccinated, not infected	84	NEG	NEG
NV2	not vaccinated, not infected	0	NEG	NEG

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NV2	not vaccinated, not infected	7	NEG	NEG
NV2	not vaccinated, not infected	14	NEG	NEG
NV2	not vaccinated, not infected	21	NEG	NEG
NV2	not vaccinated, not infected	28	NEG	NEG
NV2	not vaccinated, not infected	35	NEG	NEG
NV2	not vaccinated, not infected	42	NEG	NEG
NV2	not vaccinated, not infected	49	NEG	NEG
NV2	not vaccinated, not infected	56	NEG	NEG
NV2	not vaccinated, not infected	63	NEG	NEG
NV2	not vaccinated, not infected	70	NEG	NEG
NV2	not vaccinated, not infected	77	NEG	NEG
NV2	not vaccinated, not infected	84	NEG	NEG
V1	vaccinated, not infected	0	NEG	NEG
V1	vaccinated, not infected	7	NEG	NEG
V1	vaccinated, not infected	14	NEG	NEG
V1	vaccinated, not infected	21	POS	NEG
V1	vaccinated, not infected	28	POS	NEG
V1	vaccinated, not infected	35	POS	POS
V1	vaccinated, not infected	42	POS	NEG
V1	vaccinated, not infected	49	POS	NEG
V1	vaccinated, not infected	56	POS	NEG
V1	vaccinated, not infected	63	POS	NEG
V1	vaccinated, not infected	70	POS	NEG
V1	vaccinated, not infected	77	POS	NEG
V1	vaccinated, not infected	84	POS	NEG
V2	vaccinated, not infected	0	NEG	NEG
V2	vaccinated, not infected	7	NEG	NEG
V2	vaccinated, not infected	14	NEG	NEG
V2	vaccinated, not infected	21	NEG	NEG
V2	vaccinated, not infected	28	NEG	NEG
V2	vaccinated, not infected	35	POS	NEG
V2	vaccinated, not infected	42	POS	NEG
V2	vaccinated, not infected	49	POS	NEG
V2	vaccinated, not infected	56	POS	NEG
V2	vaccinated, not infected	63	POS	NEG
V2	vaccinated, not infected	70	POS	NEG
V2	vaccinated, not infected	77	POS	NEG
V2	vaccinated, not infected	84	POS	NEG
V3	vaccinated, not infected	0	NEG	NEG
V3	vaccinated, not infected	7	NEG	NEG
V3	vaccinated, not infected	14	NEG	NEG
V3	vaccinated, not infected	21	NEG	NEG
V3	vaccinated, not infected	28	NEG	NEG
V3	vaccinated, not infected	35	POS	NEG
V3	vaccinated, not infected	42	POS	NEG
V3	vaccinated, not infected	49	POS	NEG
V3	vaccinated, not infected	56	POS	NEG
V3	vaccinated, not infected	63	POS	NEG
V3	vaccinated, not infected	70	POS	NEG
V3	vaccinated, not infected	77	POS	NEG
V3	vaccinated, not infected	84	POS	NEG

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V4	vaccinated, not infected	0	NEG	NEG
V4	vaccinated, not infected	7	NEG	NEG
V4	vaccinated, not infected	14	POS	NEG
V4	vaccinated, not infected	21	POS	NEG
V4	vaccinated, not infected	28	POS	NEG
V4	vaccinated, not infected	35	POS	NEG
V4	vaccinated, not infected	42	POS	NEG
V4	vaccinated, not infected	49	POS	NEG
V4	vaccinated, not infected	56	POS	NEG
V4	vaccinated, not infected	63	POS	NEG
V4	vaccinated, not infected	70	POS	NEG
V4	vaccinated, not infected	77	POS	NEG
V4	vaccinated, not infected	84	POS	NEG
V5	vaccinated, not infected	0	NEG	NEG
V5	vaccinated, not infected	7	NEG	NEG
V5	vaccinated, not infected	14	NEG	NEG
V5	vaccinated, not infected	21	POS	POS
V5	vaccinated, not infected	28	POS	NEG
V5	vaccinated, not infected	35	POS	NEG
V5	vaccinated, not infected	42	POS	NEG
V5	vaccinated, not infected	49	POS	NEG
V5	vaccinated, not infected	56	POS	NEG
V5	vaccinated, not infected	63	POS	NEG
V5	vaccinated, not infected	70	POS	NEG
V5	vaccinated, not infected	77	POS	NEG
V5	vaccinated, not infected	84	POS	NEG
V5	vaccinated, not infected	0	NEG	NEG
V5	vaccinated, not infected	7	NEG	NEG
V5	vaccinated, not infected	14	NEG	NEG
V5	vaccinated, not infected	21	POS	NEG
V5	vaccinated, not infected	28	POS	NEG
V5	vaccinated, not infected	35	POS	POS
V5	vaccinated, not infected	42	POS	NEG
V5	vaccinated, not infected	49	POS	NEG
V5	vaccinated, not infected	56	POS	NEG
V5	vaccinated, not infected	63	POS	NEG
V5	vaccinated, not infected	70	POS	NEG
V5	vaccinated, not infected	77	POS	NEG
V5	vaccinated, not infected	84	POS	NEG
V7	vaccinated, not infected	0	NEG	NEG
V7	vaccinated, not infected	7	NEG	NEG
V7	vaccinated, not infected	14	NEG	NEG
V7	vaccinated, not infected	21	POS	NEG
V7	vaccinated, not infected	28	POS	NEG
V7	vaccinated, not infected	35	POS	NEG
V7	vaccinated, not infected	42	POS	POS
V7	vaccinated, not infected	49	POS	POS
V7	vaccinated, not infected	56	POS	NEG
V7	vaccinated, not infected	63	POS	NEG
V7	vaccinated, not infected	70	POS	NEG
V7	vaccinated, not infected	77	POS	NEG

V7	vaccinated, not infected	84	POS	NEG
V8	vaccinated, not infected	0	NEG	NEG
V8	vaccinated, not infected	7	NEG	NEG
V8	vaccinated, not infected	14	POS	NEG
V8	vaccinated, not infected	21	POS	NEG
V8	Vaccinated, not infected	28	POS	NEG
V8	Vaccinated, not infected	35	POS	NEG
V8	Vaccinated, not infected	42	POS	NEG
V8	Vaccinated, not infected	49	POS	NEG
V8	Vaccinated, not infected	56	POS	NEG
V8	Vaccinated, not infected	63	POS	NEG
V8	Vaccinated, not infected	70	POS	NEG
V8	Vaccinated, not infected	77	POS	NEG
V8	Vaccinated, not infected	84	POS	NEG
Inf1	Not vaccinated, infected	ND	POS	POS
Inf2	Not vaccinated, infected	ND	POS	POS
Inf3	Not vaccinated, infected	ND	POS	POS
Inf4	Not vaccinated, infected	ND	POS	POS
Inf5	Not vaccinated, infected	ND	POS	POS
Inf6	Not vaccinated, infected	ND	POS	POS
Inf7	Not vaccinated, infected	ND	POS	POS
Inf8	Not vaccinated, infected	ND	POS	POS
Inf9	Not vaccinated, infected	ND	POS	POS
inf10	Not vaccinated, infected	ND	POS	POS

Example 2

[0066] Microplate ELISA analysis was performed on serum samples collected from confirmed FIV negative and infected cats, and cats vaccinated with the FEL-O-VAX® FIV vaccine in an indirect assay format with individual FIV polypeptides on the solid phase and anti-(feline IgG) peroxidase conjugate. Antibodies to *env* FIV proteins were detected using these peptides as antigen reagents:

CELGCNQNQFFCK [SEQ ID NO:1]

CELGSNQNQFFSK [SEQ ID NO:2]

[0067] The polypeptides were synthesized using a commercial instrument and following the manufacturer's instructions. Polypeptide stocks were prepared at 5 mg/ml in DMSO. The polypeptides were then coated on microplate wells (peptide @ 10 ug/ml in 50 mM Tris-HCl pH 7.4, 100 ul/well). The plates were then

blocked/overcoated with 2% Tween-20 / 2.5% sucrose, allowed to dry in mylar bags with desiccant.

[0068] For the assays, feline serum samples (100 ul/well, diluted 1/1000 in 50% fetal bovine serum) were added to the wells and the plates were incubated for ten minutes at room temperature. Following incubation, the microplates were washed with PBS/Tween. Goat Anti-(cat IgG):peroxidase conjugate was added to the wells (100 ul/well anti-catIgG:peroxidase diluted in 50% fetal bovine serum). The plates were incubated for another fifteen minutes at room temperature and washed a second time with PBS/Tween. Peroxidase substrate was added (100 ul/well, tetramethyl benzidine peroxidase substrate) and the plates were incubated a third time for ten minutes at room temperature. A hydrofluoric acid stop solution (50 ul/well) was added to the plates. Sample antibody binding was measured by determining peroxidase activity (colored product) with a spectrophotometer (A650 nm). Significant, substantial antibody binding for a sample is considered to be A650nm greater than 0.200. The IDEXX PetChek® Anti-FIV antibody test kit was also run on these samples as a reference test. The results are shown in Table 2.

Table 2

FIV infected, not vaccinated:			
Sample	seq ID 1 A(650nm)	seq ID 2 A(650nm)	PetChek® Anti-FIV result
2689:44 7	1.862	0.908	positive
194	1.305	1.327	positive
197	1.905	0.687	positive
22488	1.943	0.802	positive
F9-1525 12/5/99	1.467	1.163	positive
23804 255	1.884	0.991	positive
F0-138 1/23/00	1.938	1.409	positive
23430 244	1.799	0.786	positive
56360 60	1.901	1.391	positive
57561 181	1.882	1.782	positive
56897 187	1.738	0.813	positive
21518	1.408	1.000	positive
58178 232	1.845	1.386	positive
23805 253	1.481	1.005	positive
21636	1.905	1.726	positive
23119	1.546	0.888	positive
55214 190	1.570	1.214	positive
57601 215	1.373	0.725	positive
21583	0.593	0.408	positive
F9-881	1.497	0.926	positive
58036 224	1.721	1.387	positive
F0-162 2/13/00	1.360	1.068	positive
57157 141	0.936	0.603	positive
23321 211	1.339	1.183	positive
56035 1	0.925	0.620	positive
mean	1.565	1.048	
FIV negative, vaccinated:			
sample	seq ID 1 A(650nm)	seq ID 2 A(650nm)	PetChek result
Vx 3495 Day 84	0.040	0.037	positive
Vx SK4 Day 84	0.043	0.038	positive
Vx 3532 Day 84	0.044	0.034	positive
Vx 3528 Day 84	0.033	0.035	positive
Vx 3519 Day 84	0.037	0.035	positive
Vx 3517 Day 84	0.050	0.034	positive
Vx SK4 Day 35	0.115	0.056	positive
Vx C3532 Day 42	0.101	0.060	positive
Vx C3519 Day 35	0.090	0.044	positive

Vx SK4 Day 21	0.052	0.035	positive
Vx C3517 Day 35	0.110	0.082	positive
mean	0.065	0.045	
FIV negative, not vaccinated:			
	seq ID 1	seq ID 2	PetChek
<u>sample</u>	<u>A(650nm)</u>	<u>A(650nm)</u>	<u>result</u>
57360 177	0.033	0.037	negative
57551 205	0.032	0.031	negative
F9-1375	0.033	0.041	negative
57208 146	0.037	0.034	negative
F9-1519 12/5/99	0.050	0.032	negative
57435 272 8/17	0.036	0.039	negative
57975 236	0.031	0.035	negative
57323 174	0.035	0.046	negative
56728 200	0.033	0.039	negative
F9-1191	0.034	0.027	negative
57528 197	0.034	0.033	negative
57095 125	0.034	0.034	negative
56704 154	0.033	0.033	negative
57911 235	0.034	0.040	negative
F9-1455 11/14/99	0.034	0.034	negative
F0-53 1/23/00	0.033	0.035	negative
57222 153	0.033	0.036	negative
56746 226	0.032	0.036	negative
57238	0.030	0.035	negative
2873	0.032	0.034	negative
22151 80	0.033	0.026	negative
57611 216	0.031	0.035	negative
57211 147	0.034	0.035	negative
F9-1211	0.034	0.033	negative
58203 230	0.031	0.033	negative
57203 145	0.033	0.032	negative
mean	0.034	0.035	

Example 3

[0069] Microplate ELISA analysis was performed as in Example 2 on serum samples collected from confirmed FIV negative and infected cats, and cats vaccinated with the FEL-O-VAX® FIV vaccine. Antibodies to FIV env were detected using these peptides as antigen reagents:

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CELGCNQNQFFCK	[SEQ ID NO:1]
KVEAMEKFLYTAFAMQELGCNQNQFFCKIPLELWTR	[SEQ ID NO:3]
TAFAMQELGSNQNQFFSK	[SEQ ID NO:4]
YTAFAMQEWGCNQNQFFCA	[SEQ ID NO:5]
TAFAMQELGCNQQQFFCA	[SEQ ID NO:6]
YTAFAMQEIGCNQNQFFCA	[SEQ ID NO:7]
CEGSNQNQFFSK	[SEQ ID NO:8]

Significant, substantial antibody binding for a sample is considered to be A650nm greater than 0.200). The results are reported in Table 3 and 4.

Table 3

FIV infected, not vaccinated:

<u>sample</u>	<u>seq ID 1</u> <u>A(650nm)</u>	<u>seq ID 3</u> <u>A(650nm)</u>	<u>seq ID 4</u> <u>A(650nm)</u>	<u>seq ID 5</u> <u>A(650nm)</u>	<u>seq ID 6</u> <u>A(650nm)</u>	<u>seq ID 7</u> <u>A(650nm)</u>	<u>PetChek</u> <u>result</u>
2689:44 7	1.862	1.911	0.908	1.696	0.893	1.153	positive
194	1.305	1.845	1.327	0.636	0.948	0.523	positive
197	1.905	1.888	0.687	1.623	1.590	1.027	positive
22488	1.943	1.714	0.802	1.162	1.663	1.296	positive
23804 255	1.884	1.115	0.991	0.829	1.078	1.036	positive
24034 283	1.508	1.423	0.663	1.436	1.318	1.202	positive
F0-138 1/23/00	1.938	1.922	1.409	1.835	1.412	1.548	positive
56360 60	1.901	2.808	1.391	1.553	1.270	1.298	positive
57561 181	1.882	1.799	1.782	1.747	1.000	1.293	positive
56897 187	1.738	1.900	0.813	1.492	0.929	1.102	positive
21518	1.408	1.319	1.000	1.419	1.032	0.955	positive
58178 232	1.845	1.952	1.386	1.338	1.392	1.237	positive
23805 253	1.481	1.205	1.005	0.562	0.721	0.599	positive
21636	1.905	1.600	1.726	1.225	1.811	1.238	positive
58232 242	1.221	1.005	0.454	1.580	0.686	1.297	positive
23119	1.546	1.446	0.888	1.154	0.447	1.156	positive
57601 215	1.373	0.985	0.725	0.987	0.722	0.601	positive
F9-881	1.497	1.951	0.926	0.587	1.272	0.589	positive
23938 323	1.489	1.203	0.635	1.475	0.999	1.333	positive
58036 224	1.721	1.855	1.387	1.218	0.828	1.102	positive
22879	1.332	1.602	0.468	1.117	0.752	1.055	positive
F0-162 2/13/00	1.360	1.118	1.068	1.284	0.702	1.046	positive
57157 141	0.936	1.089	0.603	0.491	0.415	0.417	positive
23321 211	1.339	1.057	1.183	0.751	1.308	0.886	positive
56035 1	0.925	0.776	0.620	0.368	0.691	0.508	positive
mean	1.570	1.540	0.994	1.183	1.035	1.020	

FIV negative, vaccinated:

<u>sample</u>	<u>seq ID 1</u> <u>A(650nm)</u>	<u>seq ID 3</u> <u>A(650nm)</u>	<u>seq ID 4</u> <u>A(650nm)</u>	<u>seq ID 5</u> <u>A(650nm)</u>	<u>seq ID 6</u> <u>A(650nm)</u>	<u>seq ID 7</u> <u>A(650nm)</u>	<u>PetChek</u> <u>result</u>
Vx C3520 Day 35	0.234	0.199	0.058	0.142	0.065	0.116	positive
Vx C3511 Day 49	0.208	0.115	0.155	0.209	0.111	0.180	positive
Vx C3519 Day 35	0.062	0.055	0.053	0.091	0.058	0.055	positive
Vx C3517 Day 35	0.110	0.098	0.082	0.093	0.063	0.074	positive
Vx SK4 Day 21	0.052	0.045	0.035	0.082	0.038	0.044	positive
mean	0.133	0.102	0.077	0.123	0.067	0.094	

FIV negative, not vaccinated:

<u>sample</u>	<u>seq ID 1</u> <u>A(650nm)</u>	<u>seq ID 3</u> <u>A(650nm)</u>	<u>seq ID 4</u> <u>A(650nm)</u>	<u>seq ID 5</u> <u>A(650nm)</u>	<u>seq ID 6</u> <u>A(650nm)</u>	<u>seq ID 7</u> <u>A(650nm)</u>	<u>PetChek</u> <u>result</u>
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57975 236	0.031	0.040	0.035	0.040	0.043	0.036	negative
57323 174	0.035	0.039	0.046	0.032	0.042	0.034	negative
56728 200	0.033	0.035	0.039	0.049	0.044	0.042	negative
56956 209	0.036	0.036	0.035	0.049	0.038	0.036	negative
F9-1638 143119	0.039	0.041	0.035	0.037	0.036	0.039	negative
F9-1191	0.034	0.051	0.027	0.040	0.037	0.037	negative
57528 197	0.034	0.038	0.033	0.039	0.035	0.035	negative
57095 125	0.034	0.036	0.034	0.038	0.035	0.035	negative
56704 154	0.033	0.036	0.033	0.040	0.027	0.035	negative
57911 235	0.034	0.035	0.040	0.056	0.050	0.038	negative
F9-1455 11/14/99	0.034	0.033	0.034	0.037	0.037	0.033	negative
F0-53 1/23/00	0.033	0.041	0.035	0.038	0.037	0.036	negative
57222 153	0.033	0.033	0.036	0.041	0.037	0.036	negative
56746 226	0.032	0.035	0.036	0.038	0.036	0.034	negative
F9-1278	0.034	0.035	0.024	0.038	0.036	0.034	negative
57238	0.030	0.033	0.035	0.037	0.036	0.035	negative
2873	0.032	0.040	0.034	0.036	0.033	0.034	negative
22151 80	0.033	0.032	0.026	0.039	0.036	0.047	negative
57611 216	0.031	0.031	0.035	0.067	0.043	0.044	negative
mean	0.033	0.037	0.034	0.042	0.038	0.044	

Table 4

FIV infected, not vaccinated:

	seq ID 1	seq ID 8	PetChek
<u>sample</u>	<u>A(650nm)</u>	<u>A(650nm)</u>	<u>result</u>
2426-91A	1.170	0.838	positive
2426-64-21	0.754	0.654	positive
2605	1.679	0.965	positive
21636	1.916	1.918	positive
2614	1.544	1.095	positive
3364-89	0.375	0.677	positive
58376-274	1.425	1.370	positive
Gonzalez	1.622	1.920	positive
Butzi	0.627	0.902	positive
Stanley	0.990	0.442	positive
JL-60	1.651	2.127	positive
mean	1.250	1.173	

FIV negative, vaccinated:

	seq ID 1	seq ID 8	PetChek
<u>sample</u>	<u>A(650nm)</u>	<u>A(650nm)</u>	<u>result</u>
Vx 3520 Day 84	0.040	0.040	positive
Vx 3519 Day 84	0.036	0.041	positive
Vx 3532 Day 84	0.037	0.042	positive
Vx SK4 Day 84	0.038	0.048	positive

Vx G1 week 4	0.092	0.092	positive
Vx G1 week 5	0.085	0.101	positive
Vx G1 week 6	0.091	0.111	positive
Vx G1 week 7	0.093	0.103	positive
Vx G1 week 8	0.074	0.096	positive
Vx G1 week 12	0.070	0.085	positive
mean	0.066	0.076	

FIV negative, not vaccinated:

	seq ID 1 seq ID 8 PetChek	
sample	A(650nm)	A(650nm) result
2483-83-23	0.034	0.035 negative
2483-83-30	0.035	0.035 negative
2483-83-33	0.034	0.035 negative
2151-05H	0.037	0.049 negative
768547	0.036	0.034 negative
769703	0.035	0.035 negative
768513	0.037	0.036 negative
F6263E	0.034	0.058 negative
2377-1-38	0.037	0.034 negative
14834	0.036	0.036 negative
14151	0.035	0.034 negative
D1606315	0.034	0.035 negative
mean	0.035	0.038

[0070] Although various specific embodiments of the present invention have been described herein, it is to be understood that the invention is not limited to those precise embodiments and that various changes or modifications can be affected therein by one skilled in the art without departing from the scope and spirit of the invention.

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CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 33

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NOM DU FICHER / FILE NAME :

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What is claimed is:

1. A polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
2. A method of distinguishing between animals that have been naturally infected with FIV and animals that have not been infected or have been vaccinated against an FIV infection, the method comprising:
 - contacting a biological sample from an animal with a polypeptide of SEQ ID NO:1 or claim 1;
 - detecting whether FIV antibodies in the sample substantially bind to the polypeptide;
 - determining that the animal is naturally infected by detecting that antibodies in the sample substantially bind to the FIV polypeptide and determining that the animal has been vaccinated or not infected by detecting that antibodies in the sample do not substantially bind to the FIV polypeptide.
3. The method of claim 2 wherein the biological sample is obtained from an animal that has not received an FIV vaccine within about the prior 12 weeks.
4. A method of determining whether a felid has been vaccinated against FIV or is naturally infected with FIV, the method comprising:
 - (a) detecting, before a period a time following vaccination sufficient for antibodies that are not a significant component of the animal's immune response to an FIV vaccine to be substantially eliminated from the felid, whether the felid has antibodies against an FIV peptide of SEQ ID NO:1 or claim 1;

(b) detecting, after a period a time following vaccination sufficient for antibodies that are not a significant component of an animal's immune response to the vaccine to be substantially eliminated from the felid, whether the felid has antibodies against the FIV polypeptide;

(c) determining that the felid has been successfully vaccinated by detecting antibodies in step a but not in step b, and

(d) determining that the felid is naturally infected by detecting antibodies in steps a and b.

5. A method of determining whether a felid has not been infected by FIV or has been vaccinated against FIV, the method comprising:

analyzing a biological sample from the felid to detect the presence or absence of antibodies against an FIV peptide of SEQ ID NO:1 or claim 1; and

determining that the animal has not been infected or has been or vaccinated by determining the absence of such antibodies.

6. A method of determining whether or not a felid has been vaccinated against FIV or naturally infected with FIV comprising:

providing a test device comprising a polypeptide of SEQ ID NO:1 or claim 1;

obtaining a biological sample from a felid;

running the biological sample on the test device so that the sample contacts the polypeptide; and

reading the test device, wherein a positive result indicates the cat has been naturally infected with FIV or vaccinated against FIV and wherein a negative result indicates the cat has not been naturally infected with FIV and not vaccinated against FIV.

7. A method of determining the origin of FIV polypeptide in a biological sample from an animal suspected of having a natural FIV infection or having been vaccinated against FIV, the method comprising:

obtaining a biological sample from an animal that has not been vaccinated with an FIV vaccine within about the prior twelve weeks,

contacting the biological sample obtained from the animal with:

(a) a first polypeptide that substantially binds with a significant component of the animal's antibody response to the FIV vaccine, and

(b) a polypeptide of SEQ ID NO:1 or claim 1,

detecting whether antibodies in the sample substantially bind to one or both the polypeptides; and

determining that the animal is naturally infected when the antibodies substantially bind to both peptides (a) and (b), and determining that the animal has been vaccinated when the antibodies bind to only peptide (a).

8. The method of claim 7 wherein the first polypeptide comprises FIV p15.

9. The method of claim 7 wherein the first polypeptide comprises FIV p24.

10. A diagnostic device comprising:

a dry porous carrier;

a first detection reagent immobilized on the porous carrier, said first detection reagent comprising a protein that captures FIV antibodies generated by an animal in response to either a natural FIV infection or an FIV vaccination; and

a second detection reagent immobilized on the porous carrier, said second detection reagent comprising the polypeptide of SEQ ID NO:1 or claim 1.

11. The diagnostic device according to claim 10, wherein the first detection reagent comprises FIV p15.
12. The diagnostic device according to claim 10, wherein the first detection reagent comprises FIV p24.
13. A method for determining whether a felid has been vaccinated against FIV or is naturally infected with FIV, the method comprising contacting a biological sample with the diagnostic device of claim 10 and detecting whether antibodies in the sample substantially bind one or both of the first detection reagent and the second detection reagent.
14. A diagnostic device comprising:
 - a first porous carrier comprising a first detection reagent immobilized thereon; said first detection reagent comprising a polypeptide that captures FIV antibodies generated by an animal in response to either a natural FIV infection or an FIV vaccination; and
 - a second porous carrier comprising a second detection reagent immobilized thereon, said second detection reagent comprising a polypeptide of SEQ ID NO:1 or claim 1.
15. The diagnostic device according to claim 14, wherein the first detection reagent comprises FIV p15.
16. The diagnostic device according to claim 14, wherein the first detection reagent comprises FIV p24.
17. A method of determining the vaccination status of a felid comprising:
 - providing the polypeptide of SEQ ID NO:1 or claim 1;

contacting a biological sample from a felid with the polypeptide to form a polypeptide/antibody complex; and

determining the presence or absence of the polypeptide/antibody complex, wherein the presence of the complex represents natural infection and the absence of the complex represents either vaccination or no infection.

18. The method of claim 17 wherein the biological sample is obtained from an animal that has not received an FIV vaccine within about the prior 12 weeks.

19. A method of determining whether a felid has been vaccinated against FIV or is naturally infected with FIV comprising determining the felid's immune response to the polypeptide of SEQ ID NO:1 or claim 1.

20. Use of a polypeptide derived from FIV *env* in the manufacture of a kit for the diagnosis of whether a cat has been vaccinated against FIV.

21. Use, as claimed in claim 20, wherein the method also involves determining whether the cat has been infected with FIV.

22. The polypeptide as claimed in claim 1, wherein the polypeptide comprises antigenic fragments and functionally-equivalents thereof.

23. The polypeptide as claimed in claim 1, wherein the polypeptide is in the form of a fusion protein comprising an additional polypeptide fused to said amino acid sequence, or which is coupled to a carrier protein or polypeptide.

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CLAIMS

1. A polypeptide selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
2. A method of distinguishing between animals that have been naturally infected with FIV and animals that have not been infected or have been vaccinated against an FIV infection, the method comprising:
 - contacting a biological sample from an animal with a polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;
 - detecting whether FIV antibodies in the sample substantially bind to the polypeptide;
 - determining that the animal is naturally infected by detecting that antibodies in the sample substantially bind to the FIV polypeptide and determining that the animal has been vaccinated or not infected by detecting that antibodies in the sample do not substantially bind to the FIV polypeptide.
3. The method of claim 2 wherein the biological sample is obtained from an animal that has not received an FIV vaccine within about the prior 12 weeks.
4. A method of determining whether a felid has been vaccinated against FIV or is naturally infected with FIV, the method comprising:
 - (a) detecting, before a period a time following vaccination sufficient for antibodies that are not a significant component of the animal's immune response to an FIV vaccine to be substantially eliminated from the felid, whether the felid has antibodies against an FIV polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;

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(b) detecting, after a period a time following vaccination sufficient for antibodies that are not a significant component of an animal's immune response to the vaccine to be substantially eliminated from the felid, whether the felid has antibodies against the FIV polypeptide;

(c) determining that the felid has been successfully vaccinated by detecting antibodies in step a but not in step b, and

(d) determining that the felid is naturally infected by detecting antibodies in steps a and b.

5. A method of determining whether a felid has not been infected by FIV or has been vaccinated against FIV, the method comprising:

analyzing a biological sample from the felid to detect the presence or absence of antibodies against an FIV polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8; and

determining that the animal has not been infected or has been or vaccinated by determining the absence of such antibodies.

6. A method of determining whether or not a felid has been vaccinated against FIV or naturally infected with FIV comprising:

providing a test device comprising a polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;

obtaining a biological sample from a felid;

running the biological sample on the test device so that the sample contacts the polypeptide; and

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reading the test device, wherein a positive result indicates the cat has been naturally infected with FIV and wherein a negative result indicates the cat has not been naturally infected with FIV or vaccinated against FIV.

7. A method of determining the origin of FIV polypeptide in a biological sample from an animal suspected of having a natural FIV infection or having been vaccinated against FIV, the method comprising:

obtaining a biological sample from an animal that has not been vaccinated with an FIV vaccine within about the prior twelve weeks,

contacting the biological sample obtained from the animal with:

(a) a first polypeptide that substantially binds with a significant component of the animal's antibody response to the FIV vaccine, and

(b) a polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8,

detecting whether antibodies in the sample substantially bind to one or both the polypeptides; and

determining that the animal is naturally infected when the antibodies substantially bind to both peptides (a) and (b), and determining that the animal has been vaccinated when the antibodies bind to only peptide (a).

8. The method of claim 7 wherein the first polypeptide comprises FIV p15.

9. The method of claim 7 wherein the first polypeptide comprises FIV p24.

10. A diagnostic device comprising:
a dry porous carrier;

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a first detection reagent immobilized on the porous carrier at a first location, said first detection reagent comprising a protein that captures FIV antibodies generated by an animal in response to either a natural FIV infection or an FIV vaccination; and

a second detection reagent immobilized on the porous carrier at a second location different from the first location, said second detection reagent comprising the polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

11. The diagnostic device according to claim 10, wherein the first detection reagent comprises FIV p15.

12. The diagnostic device according to claim 10, wherein the first detection reagent comprises FIV p24.

13. A method for determining whether a felid has been vaccinated against FIV or is naturally infected with FIV, the method comprising contacting a biological sample with the diagnostic device of claim 10 and detecting whether antibodies in the sample substantially bind one or both of the first detection reagent and the second detection reagent.

14. A diagnostic device comprising:

a first porous carrier comprising a first detection reagent immobilized thereon; said first detection reagent comprising a polypeptide that captures FIV antibodies generated by an animal in response to either a natural FIV infection or an FIV vaccination; and

a second porous carrier comprising a second detection reagent immobilized thereon, said second detection reagent comprising a polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

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15. The diagnostic device according to claim 14, wherein the first detection reagent comprises FIV p15.
16. The diagnostic device according to claim 14, wherein the first detection reagent comprises FIV p24.
17. A method of determining the vaccination status of a felid comprising:
providing the polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;
contacting a biological sample from a felid with the polypeptide to form a polypeptide/antibody complex; and
determining the presence or absence of the polypeptide/antibody complex, wherein the presence of the complex represents natural infection and the absence of the complex represents either vaccination or no infection.
18. The method of claim 17 wherein the biological sample is obtained from an animal that has not received an FIV vaccine within about the prior 12 weeks.
19. A method of determining whether a felid has been vaccinated against FIV or is naturally infected with FIV comprising determining the felid's immune response to a polypeptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
20. Use of a polypeptide derived from FIV *env* in the manufacture of a kit for the diagnosis of whether a cat has been vaccinated against FIV.

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21. Use, as claimed in claim 20, wherein the method also involves determining whether the cat has been infected with FIV.
22. The polypeptide as claimed in claim 1, wherein the polypeptide is in the form of a fusion protein comprising an additional polypeptide fused to said amino acid sequence, or which is coupled to a carrier protein or polypeptide.