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CA 2579676 A1 2007/02/15

(21) 2 579 676

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2005/09/02
(87) Date publication PCT/PCT Publication Date: 2007/02/15
(85) Entrée phase nationale/National Entry: 2007/03/06
(86) N° demande PCT/PCT Application No.: US 2005/031287
(87) N° publication PCT/PCT Publication No.: 2007/018550
(30) Priorités/Priorities: 2004/09/08 (US60/607,579);
2005/05/03 (US60/676,931)

(51) Cl.Int./Int.Cl. *G01N 33/50* (2006.01)

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THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF HEALTH AND HUMAN SERVICES, NIH OFFICE OF TECHNOLOGY TRANSFER, US

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(54) Titre : COMPOSITIONS ET METHODES POUR DETECTER UNE INFECTION PAR LE VIH-1/VIH-2
(54) Title: COMPOSITIONS AND METHODS FOR THE DETECTION OF HIV-1/HIV-2 INFECTION

(57) Abrégé/Abstract:
This invention relates to compositions and methods or the detection of immunodeficiency virs infection, especially immunodeficiency virus-1 (HIV-1) infection. The invention particularly concerns compositions and methods that may be used in HIV vaccine recipients whose sera may contain vaccine-generated anti-HIV-1 antibodies.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2007 (15.02.2007)

PCT

(10) International Publication Number
WO 2007/018550 A2

(51) International Patent Classification:
G01N 33/50 (2006.01)

(21) International Application Number:
PCT/US2005/031287

(22) International Filing Date:
2 September 2005 (02.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/607,579	8 September 2004 (08.09.2004)	US
60/676,931	3 May 2005 (03.05.2005)	US

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(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, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, 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, LV, 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:

— *without international search report and to be republished upon receipt of that 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.

WO 2007/018550 A2

(54) Title: COMPOSITIONS AND METHODS FOR THE DETECTION OF HIV-1/HIV-2 INFECTION

(57) Abstract: This invention relates to compositions and methods or the detection of immunodeficiency virs infection, especially immunodeficiency virus-1 (HIV-1) infection. The invention particularly concerns compositions and methods that may be used in HIV vaccine recipients whose sera may contain vaccine-generated anti-HIV-1 antibodies.

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THIS IS VOLUME __1___ OF __2___

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Title of the Invention

Compositions And Methods For The Detection Of HIV-1/HIV-2 Infection

Cross-Reference to Related Applications

This application claims priority to United States Patent Applications Serial Nos. 60/676,931 (filed on May 3, 2005) and 60/607,579 (filed on September 8, 2004), which applications are each herein incorporated by reference in their entirety.

Field of the Invention

This invention relates to compositions and methods for the detection of immunodeficiency virus infection, especially human immunodeficiency virus-1 (HIV-1) infection. The invention particularly concerns compositions and methods that may be used in HIV vaccine recipients whose sera may contain vaccine-generated anti-HIV-1 antibodies.

Background of the Invention

The human immunodeficiency virus (HIV) is a pathogenic retrovirus (Varmus, H. (1988) "RETROVIRUSES," Science 240:1427-1439; Cowley S. (2001) "THE BIOLOGY OF HIV INFECTION" Lepr Rev. 72(2):212-20). HIV-1 is the causative agent of acquired immune deficiency syndrome (AIDS) and related disorders (Gallo, R.C. *et al.* (1983) "Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS)," Science 220(4599):865-7; Barre-Sinoussi, F. *et al.* "ISOLATION OF A T-LYMPHOTROPIC RETROVIRUS FROM A PATIENT AT RISK FOR ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)," (1983) Science 220:868-870; Gallo, R. *et al.* (1984) "FREQUENT DETECTION AND ISOLATION OF CYTOPATHIC RETROVIRUSES (HTLV-III) FROM PATIENTS WITH AIDS AND AT RISK FOR AIDS," Science 224:500-503; Teich, N. *et al.* (1984) "RNA TUMOR VIRUSES," Weiss, R. *et al.* (eds.) Cold Spring Harbor Press (NY) pp. 949-956).

Since 1987, more than 25,000 individuals have received immunizations with human immunodeficiency virus (HIV) preventive vaccines. Currently, most of the HIV vaccine candidates are complex products containing multiple viral genes or proteins. Prime-boost strategies are under way to optimize cellular and humoral immune responses. Consequently, vaccine recipients' sera are often reactive in licensed HIV serodetection assays, generating patterns indistinguishable from HIV-infected individuals (Ackers, M.L. *et al.* (2003) "HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVITY AMONG UNINFECTED HIV VACCINE RECIPIENTS," J Infect Dis 187:879-986; Pitisuttithum, P. *et al.* (2003) "SAFETY AND IMMUNOGENICITY OF COMBINATIONS OF RECOMBINANT SUBTYPE E AND B HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ENVELOPE GLYCOPROTEIN 120 VACCINES IN HEALTHY THAI ADULTS," J Infect Dis 188:219-227; Chuenchitra, T. *et al.* (2003) "LONGITUDINAL STUDY OF HUMORAL IMMUNE RESPONSES IN HIV TYPE 1 SUBTYPE CRF01_AE (E)-INFECTED THAI PATIENTS WITH DIFFERENT RATES OF DISEASE PROGRESSION," AIDS Res Hum Retroviruses 19:293-305; Schwartz, D.H. *et al.* (1995) "UTILITY OF VARIOUS COMMERCIALLY AVAILABLE HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY DIAGNOSTIC KITS FOR USE IN CONJUNCTION WITH EFFICACY TRIALS OF HIV-1 VACCINES," Clin Diagn Lab Immunol 2, 268-271). This will have a negative impact on future prophylactic vaccine trials, in which early detection of HIV infections is of paramount importance. Furthermore, long-term HIV seropositivity will exclude vaccine trial participants from the pool of blood and plasma donors, and will contribute to a plethora of socio-economic harms including denied employment, health insurance, travel, immigration, and recruitment to the armed forces (Belshe, R.B. *et al.* (1994) "INTERPRETING HIV SERODIAGNOSTIC TEST RESULTS IN THE 1990S: SOCIAL RISKS OF HIV VACCINE STUDIES IN UNINFECTED VOLUNTEERS," NIAID AIDS Vaccine Clinical Trials Group. Ann Intern Med 121:584-589; Allen, M. *et al.* (2001) "TRIAL-RELATED DISCRIMINATION IN HIV VACCINE CLINICAL TRIALS. AIDS RES HUM RETROVIRUSES," 17:667-674). Therefore, the prospect of seroconversion could deter potential trial participants and severely curtail recruitment into large scale trials around the globe (Gross, M. *et al.* (1996) "INTEREST AMONG

GAY/BISEXUAL MEN IN GREATER BOSTON IN PARTICIPATING IN CLINICAL TRIALS OF PREVENTIVE HIV VACCINES," J Acquir Immune Defic Syndr Hum Retrovirol 12:406-412; Sheon, A.R. *et al.* (1998) "PREVENTING DISCRIMINATION AGAINST VOLUNTEERS IN PROPHYLACTIC HIV VACCINE TRIALS: LESSONS FROM A PHASE II TRIAL," J Acquir Immune Defic Syndr Hum Retrovirol 19:519-526; Koblin, B.A. *et al.* (1998) "READINESS OF HIGH-RISK POPULATIONS IN THE HIV NETWORK FOR PREVENTION TRIALS TO PARTICIPATE IN HIV VACCINE EFFICACY TRIALS IN THE UNITED STATES," Aids 12:785-793. Currently, there is no HIV detection assay that differentiates between vaccine generated antibodies and those produced after true HIV infection during HIV vaccine trials.

HIV-2 (also known as the West African AIDS Virus) is closely related to the simian immunodeficiency virus, and infected individuals are found primarily in West Africa (Smith, R.S. *et al.* (1990) "SYNTHETIC PEPTIDE ASSAYS TO DETECT HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 IN SEROPOSITIVE INDIVIDUALS," Arch Pathol Lab Med. 114(3):254-258; Baillou, A. *et al.* (1991) "FINE SEROTYPING OF HUMAN IMMUNODEFICIENCY VIRUS SEROTYPE 1 (HIV-1) AND HIV-2 INFECTIONS BY USING SYNTHETIC OLIGOPEPTIDES REPRESENTING AN IMMUNODOMINANT DOMAIN OF HIV-1 AND HIV-2/SIMIAN IMMUNODEFICIENCY VIRUS," J Clin Microbiol. 29(7):1387-1391).

HIV acts to compromise the immune system of infected individuals by targeting and infecting the CD-4$^+$ T lymphocytes that would otherwise be the major proponents of the recipient's cellular immune system response (Dalgleish, A. *et al.* (1984) "THE CD4 (T4) ANTIGEN IS AN ESSENTIAL COMPONENT OF THE RECEPTOR FOR THE AIDS RETROVIRUS," Nature 312: 767-768, Maddon *et al.* (1986) "THE T4 GENE ENCODES THE AIDS VIRUS RECEPTOR AND IS EXPRESSED IN THE IMMUNE SYSTEM AND THE BRAIN," Cell 47:333-348; McDougal, J. S. *et al.* (1986) "BINDING OF HTLV-III/LAV TO T4+ T CELLS BY A COMPLEX OF THE 110K VIRAL PROTEIN AND THE T4 MOLECULE," Science 231:382-385). HIV infection is pandemic and HIV-associated diseases represent a major world health problem.

Infection of cells by HIV -1 requires membrane attachment of the virion and subsequent fusion of the viral and cellular membranes. The fusion process is mediated by the viral outer envelope glycoprotein complex (gp120/gp41) and target cell receptors (McGaughey, G.B. *et al.* (2004) "PROGRESS TOWARDS THE DEVELOPMENT OF A HIV-1 GP41-DIRECTED VACCINE," Curr HIV Res. 2(2):193-204). The envelope glycoprotein is synthesized as a precursor protein (gp160) that is proteolytically cleaved into two non-covalently associated protein subunits, a surface subunit (gp120) and a transmembrane subunit (gp41). The gpl20 envelope protein is responsible for binding to the CD4 cell-surface receptor and a chemokine co-receptor, CCR5 or CXCR4. Following receptor binding, the membrane-anchored gp41 mediates fusion of the viral and target cell membranes. The gp41 ectodomain contains a hydrophobic, glycine-rich fusion peptide (amino acids 512-527) at the amino terminus that is essential for membrane fusion (numbering based on HXB2 gp160 variant as described in Chan, D.C. *et al.* (1997) "CORE STRUCTURE OF GP41 FROM THE HIV ENVELOPE GLYCOPROTEIN," Cell 89(2):263-273). Two 4,3 hydrophobic repeat regions following the fusion peptide are defined by a heptad repeat (abcdefg)n, where the residues occupying the a and d positions are predominantly hydrophobic. The two heptad repeat regions are referred to as the N36 (residues 546-581) and C34 (residues 628-661) peptides. A loop region containing a disulfide linkage separates the two heptad repeat regions. The region of the gp41 ectodomain proximal to the viral membrane is abundant in the amino acid tryptophan (amino acids 665¬683) and has been shown to be critical for the membrane fusion mechanism of HIV-1. Gp4l exists in two distinct conformations, a native or non-fusogenic state and a fusion-active state (fusogenic state) (McGaughey, G.B. *et al.* (2004) "PROGRESS TOWARDS THE DEVELOPMENT OF A HIV-1 GP41-DIRECTED VACCINE," Curr HIV Res. 2(2):193-204). On the surface of free virions, gp41 exists in the native state with the N-terminal fusion peptide largely inaccessible. Following interaction of the gp120/gp41 complex with cell-surface receptors, gp41 undergoes a series of conformational changes leading to the fusion-active conformation (Chan, D.C. *et al.* (1998) HIV ENTRY AND ITS INHIBITION," Cell 93(5):681-684). The transition from the native non-fusogenic to

fusion-competent state proceeds through a nascent species termed the prehairpin intermediate. In this transient conformation, the N- and C-tenninal regions of gp41 become separated; the N-terminal fusion peptide is inserted into the target cell membrane and the C-terminal region is anchored to the viral membrane. The prehairpin intermediate ultimately folds into the fusion-active confonnation bringing the viral and target membranes into proximity allowing viral entry into the target cell (Chan, D.C. *et al.* (1998) HIV ENTRY AND ITS INHIBITION," Cell 93(5):681-684).

The detection of HIV infection may be accomplished by either identifying viral proteins in the sera of infected individuals, by identifying viral nucleic acids in plasma or cells, or by detecting host antibodies that are produced by such individuals in response to viral infection. Strategies involving the detection of viral proteins are complicated by the low levels of such proteins during HIV infection, and by high assay cost. Thus, the detection of HIV infection is typically accomplished by detecting host anti-HIV antibodies (Manocha, M. *et al.* (2003) "COMPARING MODIFIED AND PLAIN PEPTIDE LINKED ENZYME IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) AND TYPE-2 (HIV-2) ANTIBODIES," Immunol Lett. 85(3):275-278). Such detection is however, complicated by the etiology of HIV infection, in which a significant initial "eclipse" period precludes detection of elicited antibodies (Mortimer, P.P. (1991) "THE FALLIBILITY OF HIV WESTERN BLOT," Lancet 11:286-286), and by persistant false positive results (Gnann, J.W. Jr. *et al.* (1989) "CUSTOM-DESIGNED SYNTHETIC PEPTIDE IMMUNOASSAYS FOR DISTINGUISHING HIV TYPE 1 AND TYPE 2 INFECTIONS," *Methods Enzymol.* 178:693-714). Due to these problems, more sensitive and expensive tests, such as the Western blot are often needed to confirm positive screening test results or to detect low level of circulating virus. However, Western blot analyses sometimes give indeterminate results so that a combination of screening tests (ELISA or Rapid tests) is required to confirm the diagnosis (Mortimer, P.P. (1991) "THE FALLIBILITY OF HIV WESTERN BLOT," Lancet 11:286-286; Brattegaard, K. *et al.* (1995) "INSENSITIVITY OF A SYNTHETIC PEPTIDE-BASED TEST (PEPTI-LAV 1-2) FOR THE DIAGNOSIS OF

HIV INFECTION IN AFRICAN CHILDREN," *AIDS* 9(6):656-657). Additionally, different HIV proteins are expressed at different times during infection. For example, the env-gene products of HIV have been found to induce an immune response that precedes the immune response of HIV's gag-related gene products (Döpel, S.H. *et al.* (1991) "COMPARISON OF FOUR ANTI-HIV SCREENING ASSAYS WHICH BELONG TO DIFFERENT TEST GENERATIONS," *Eur. J. Clin. Chem. Clin. Biochem.* 29:331-337).

The most common screening method for the diagnosis of infection with human immunodeficiency virus (HIV) is the detection (by sandwich ELISA) of virus-specific antibodies elicted by infected individuals in response to the infection (Döpel, S.H. *et al.* (1991) "COMPARISON OF FOUR ANTI-HIV SCREENING ASSAYS WHICH BELONG TO DIFFERENT TEST GENERATIONS," Eur. J. Clin. Chem. Clin. Biochem. 29:331-337; Manocha, M. *et al.* (2003) "COMPARING MODIFIED AND PLAIN PEPTIDE LINKED ENZYME IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) AND TYPE-2 (HIV-2) ANTIBODIES," *Immunol Lett.* 85(3):275-278; Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," *Curr Protein Pept Sci.* 4(4):285-290; Beristain, C.N. *et al.* (1995) "EVALUATION OF A DIPSTICK METHOD FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION," *J. Clin. Lab. Anal.* 9(6):347-350). "First generation" assays used purified viral proteins obtained from infected cells to bind, and identify, such antibodies. However, since diagnostically relevant viral proteins, such as those encoded by the HIV-1 env gene were difficult to obtain in large quantities, "second generation"assays were soon developed that employed recombinantly produced HIV antigens.

Unfortunately, the use of such recombinant products requires extensive protein purification in order to avoid false positive results. Thus, it has been proposed that synthetic peptides be used to bind to and detect HIV-1 antibodies (Döpel, S.H. *et al.* (1991) "COMPARISON OF FOUR ANTI-HIV SCREENING ASSAYS WHICH BELONG TO DIFFERENT TEST GENERATIONS," Eur. J. Clin. Chem. Clin.

Biochem. 29:331-337; Manocha, M. *et al.* (2003) "COMPARING MODIFIED AND PLAIN PEPTIDE LINKED ENZYME IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) AND TYPE-2 (HIV-2) ANTIBODIES," Immunol Lett. 85(3):275-278; Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 4(4):285-290; Baillou, A. *et al.* (1991) "FINE SEROTYPING OF HUMAN IMMUNODEFICIENCY VIRUS SEROTYPE 1 (HIV-1) AND HIV-2 INFECTIONS BY USING SYNTHETIC OLIGOPEPTIDES REPRESENTING AN IMMUNODOMINANT DOMAIN OF HIV-1 AND HIV-2/SIMIAN IMMUNODEFICIENCY VIRUS," J Clin Microbiol. 29(7):1387-1391; Gnann, J.W. Jr. *et al.* (1989) "CUSTOM-DESIGNED SYNTHETIC PEPTIDE IMMUNOASSAYS FOR DISTINGUISHING HIV TYPE 1 AND TYPE 2 INFECTIONS," Methods Enzymol. 178:693-714; Beristain, C.N. *et al.* (1995) "EVALUATION OF A DIPSTICK METHOD FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION," J Clin Lab Anal. 1995;9(6):347-350; Modrow, S. *et al.* (1989) "CARRIER BOUND SYNTHETIC OLIGOPEPTIDES IN ELISA TEST SYSTEMS FOR DISTINCTION BETWEEN HIV-1 AND HIV-2 INFECTION," J Acquir Immune Defic Syndr. 2(2):141-148; Smith, R.S. *et al.* (1990) "SYNTHETIC PEPTIDE ASSAYS TO DETECT HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 IN SEROPOSITIVE INDIVIDUALS," Arch Pathol Lab Med. 114(3):254-258).

Synthetic peptide antigens coupled with ELISA offers several potential advantages over other types of assays, potentially increasing the sensitivity and specificity of the assay, decreasing its cost, and providing a relatively simple format that would be suitable for testing sizeable number of samples in any laboratory (Manocha, M. *et al.* (2003) "COMPARING MODIFIED AND PLAIN PEPTIDE LINKED ENZYME IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) AND TYPE-2 (HIV-2) ANTIBODIES," Immunol Lett. 85(3):275-278). Additionally, such peptides, if they elicit antibody formation, could be used as an anti-HIV vaccine (Petrov, R.V. *et al.* (1990) "THE USE OF SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTIONS," Biomed Sci. 1(3):239-244).

Suitable synthetic peptides comprise short protein sequences that can be recognized by antibodies that have been elicited through an individual's exposure to the intact viral protein (Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 4(4):285-290). In particular, it has been proposed that such peptides must possess the following characteristics: (1) an ability to detect an antibody response in all HIV-infected individuals; (2) an ability to detect an antibody response as early as possible after infection; and (3) an ability to maintain detection of antibody response over all stages of the disease (Döpel, S.H. *et al.* (1991) "COMPARISON OF FOUR ANTI-HIV SCREENING ASSAYS WHICH BELONG TO DIFFERENT TEST GENERATIONS," Eur. J. Clin. Chem. Clin. Biochem. 29:331-337; Döpel, S.H. *et al.* (1990) "FINE MAPPING OF AN IMMUNODOMINANT REGION OF THE TRANSMEMBRANE PROTEIN OF THE HUMAN IMMUNODEFICIENCY VIRUS (HIV-1)," J. Virol. Meth. 25:167-178; Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 4(4):285-290). In particular, the HIV-1 p24 (gag) protein, gp160/120 (env) protein and gp41 (env envelope transmembrane protein) have been proposed as having serodiagnostic importance, and as being a potential source of suitable peptides (Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 4(4):285-290).

Additionally, it is important to be able to distinguish between the HIV-1 and HIV-2 variants of HIV (Smith, R.S. *et al.* (1990) "SYNTHETIC PEPTIDE ASSAYS TO DETECT HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 IN SEROPOSITIVE INDIVIDUALS," Arch Pathol Lab Med. 114(3):254-258; Baillou, A. *et al.* (1991) "FINE SEROTYPING OF HUMAN IMMUNODEFICIENCY VIRUS SEROTYPE 1 (HIV-1) AND HIV-2 INFECTIONS BY USING SYNTHETIC OLIGOPEPTIDES REPRESENTING AN IMMUNODOMINANT DOMAIN OF HIV-1 AND HIV-2/SIMIAN IMMUNODEFICIENCY VIRUS," J Clin Microbiol. 29(7):1387-1391; Modrow, S. *et al.* (1989) "CARRIER BOUND SYNTHETIC OLIGOPEPTIDES IN ELISA TEST SYSTEMS FOR DISTINCTION BETWEEN HIV-1 AND HIV-2 INFECTION," J Acquir Immune Defic Syndr. 2(2):141-148). These variants share 40-60% homology at the amino acid level (Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION,"

Curr Protein Pept Sci. 4(4):285-290; Gueye-Ndiaye, A. *et al.* (1993) "COST-EFFECTIVE DIAGNOSIS OF HIV-1 AND HIV-2 BY RECOMBINANT-EXPRESSED ENV PEPTIDE (566/996) DOT-BLOT ANALYSIS," AIDS. 7(4):475-481).

Methods for detecting HIV or other viral pathogens are disclosed in WO9008162A1, and in United States Patents Nos: 6,689,879; 6,649,749; 6,623,920; 6,589,734; 6,586,177; 6,582,920; 6,541,609; 6,534,285; 6,531,276; 6,492 104; 6,458,527; 6,399,307; 6,352,826; 6,270,959; 6,252,059; 6,245,737; 6,048,685; 6,008,044; 5,928,642; 5,925,513; 5,891,623; 5,856,088; 5,849,494; 5,830,641; 5,721,095; 5,712,385; 5,705,331; 5,695,930; 5,681,696; 5,660,979; 5,637,453; 5,599,662; 5,476,765; 5,462,852; 5,459,060; 5,260,189; and in European Patent Documents EP0439077B1, EP0439077A2.

Substantial progress has been made in the management and treatment of HIV-1 infection. However, available antiretroviral therapies can cause metabolic toxicity (Sommerfelt, M.A. *et al.* (2004) "NOVEL PEPTIDE-BASED HIV-1 IMMUNOTHERAPY," Expert Opin Biol Ther. 4(3):349-61), and thus alternative strategies to control HIV-1 infection are needed (McGaughey, G.B. *et al.* (2004) "PROGRESS TOWARDS THE DEVELOPMENT OF A HIV-1 GP41-DIRECTED VACCINE," Curr HIV Res. 2(2):193-204). The use of peptide immunogens has been proposed as the basis for an anti-HIV-1 vaccine (Sommerfelt, M.A. *et al.* (2004) "NOVEL PEPTIDE-BASED HIV-1 IMMUNOTHERAPY," Expert Opin Biol Ther. 4(3):349-61; Berzofsky, J.A. *et al.* (1995) "NOVEL APPROACHES TO PEPTIDE AND ENGINEERED PROTEIN VACCINES FOR HIV USING DEFINED EPITOPES: ADVANCES IN 1994-1995," AIDS 9 Suppl A:S143-157; Choppin, J. *et al.* (2001) "CHARACTERISTICS OF HIV-1 NEF REGIONS CONTAINING MULTIPLE CD8+ T CELL EPITOPES: WEALTH OF HLA-BINDING MOTIFS AND SENSITIVITY TO PROTEASOME DEGRADATION," J Immunol. 166(10):6164-6169; Berzofsky, J.A. *et al.* (1999) "APPROACHES TO IMPROVE ENGINEERED VACCINES FOR HUMAN IMMUNODEFICIENCY VIRUS AND OTHER VIRUSES THAT CAUSE CHRONIC INFECTIONS," Immunol Rev. 170:151-172; Cho, M.W. (2003) "SUBUNIT PROTEIN VACCINES: THEORETICAL AND PRACTICAL CONSIDERATIONS FOR HIV-1," Curr Mol Med. 3(3):243-263; Sabatier, J.M. *et al.*

(1989) "USE OF SYNTHETIC PEPTIDES FOR THE DETECTION OF ANTIBODIES AGAINST THE NEF REGULATING PROTEIN IN SERA OF HIV-INFECTED PATIENTS," AIDS. 3(4):215-220; van der Ryst, E. (2002) "PROGRESS IN HIV VACCINE RESEARCH," Oral Dis. 8 Suppl 2:21-26; Zolla-Pazner, S. (2004) "IDENTIFYING EPITOPES OF HIV-1 THAT INDUCE PROTECTIVE ANTIBODIES," Nat Rev Immunol. 4(3):199-210.

Unfortunately, the identification of suitable peptides is encumbered by the rapid mutation and recombination exhibited by retroviruses, extreme variability is found in HIV proteins. Although conserved regions in HIV-1 gp120 (residues 495-516), gp41 (residues 67-83 and 584-618), and gp36 (residues 574-602) have been investigated as potential sequences for candidate peptides (Alcaro, M.C. *et al.* (2003) "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 4(4):285-290), prior efforts to define suitable peptides have not been fully satisfactory. Petrov, R.V. *et al.* disclose that many candidate peptides failed to identify HIV infection in HIV-infected individuals, necessitating the use of multiple peptides in order to detect HIV infection (Petrov, R.V. *et al.* (1990) "THE USE OF SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTIONS," Biomed Sci. 1(3):239-244). Thus, an important problem facing the field of HIV diagnostics is the identification of a suitable peptide that would be recognized broadly, or universally, by the full range of clinically identified HIV variants. Likewise, at present no identified peptide has resulted in an HIV-1 immunotherapy that could be used as the basis for a vaccine that would provide substantial or full immunoprotection to infection by such variants (Sommerfelt, M.A. *et al.* (2004) "NOVEL PEPTIDE-BASED HIV-1 IMMUNOTHERAPY," Expert Opin Biol Ther. 4(3):349-61). In addition, suitable diagnostic tests should be able to distinguish between individuals whose sera contain anti-HIV antibodies as a result of their receipt of an anti-HIV vaccine and individuals whose sera contain anti-HIV antibodies as a result of HIV infection. The present invention is directed to this and other needs.

Summary of the Invention

This invention relates to compositions and methods for the detection of immunodeficiency virus infection, especially human immunodeficiency virus-1 (HIV-1) and human immunodeficiency virus-2 (HIV-2) infection. The invention particularly concerns compositions and methods that may be used in HIV vaccine recipients whose sera may contain vaccine-generated anti-HIV antibodies. The invention also concerns peptide antigens that may be used in anti-HIV vaccine compositions.

Most of the HIV-1 prophylactic vaccines currently under development are complex products containing multiple viral genes or proteins. As a result, vaccine recipients' sera are expected to be identified as reactive in HIV-1 or HIV-2 or both HIV 1 & 2 seroconversion detection assays and thus to produce patterns indistinguishable form sera obtained from infected individuals. This will have a negative impact on future clinical trials of prophylactic HIV vaccines that require early detection of breakthrough infections. It will also exclude all vaccinees from the pool of potential blood donors, and may contribute to other social harms. The present invention is directed, in part, to the identification of new HIV-1 and HIV-2 epitopes which are: (a) broadly reactive with early serum samples from individuals infected with HIV virus strains from all clades; (b) do not contain protective antibody or cytotoxic epitopes; and (c) can be easily removed from current and future HIV-1 candidates. In a preferred embodiment, Gene-Fragment Phage Display libraries constructed from whole HIV-1 genome are used to identify such epitopes and to construct differential enzyme-immunoassays that are capable of distinguishing reactivities from infection-induced anti-HIV antibodies from vaccine-induced anti-HIV reactivities.

In one aspect, the invention relates to a method for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of: (a) contacting said biological sample with a peptide having an epitope that is recognized by said anti-HIV-1 antibody, said contacting

being under conditions sufficient to permit said anti-HIV-1 antibody if present in said sample to bind to said epitope and form a peptide-anti-HIV-1 antibody complex; (b) contacting said formed peptide-anti-HIV-1 antibody complex with an anti-HIV-1 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody binding molecule to bind to anti-HIV-1 antibody of said formed peptide-anti-HIV-1 antibody complex and form an extended complex; and (c) determining the presence or concentration of said anti-HIV-1 antibody in said biological sample by determining the presence or concentration of said formed extended complex; wherein said epitope is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, 90** and **141.**

In another aspect, the invention relates to a a peptide or protein comprising an epitope that is recognized by an anti-HIV-1 antibody, wherein said epitope is present on a peptide having the amino acid sequence of **SEQ ID NO:3, SEQ ID NO:50, SEQ ID NO:55** or **SEQ ID NO:141.**

In another aspect, the invention relates to a peptide or protein having the amino acid sequence of **SEQ ID NO:3, SEQ ID NO:50, SEQ ID NO:55** or **SEQ ID NO:141**.

In another aspect, the invention relates to an immunological complex comprising a peptide bound to an anti-HIV-1 antibody, wherein said anti-HIV-1 antibody is additionally bound to an anti-HIV antibody binding molecule, wherein said peptide or protein comprises an epitope that is recognized by an anti-HIV-1 antibody, said epitope being present on a peptide or protein having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, 90** and **141**.

In a further aspect, the invention relates to a kit for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said kit comprises a hollow casing comprising a multilayer filter system, and first and second porous carriers, wherein said second porous

carrier is in communication with said first porous carrier, and said first porous carrier is in communication with said multilayer filter system, a portion of which is accessible from said casing; wherein said first porous carrier contains a non-immobilized, labeled peptide or protein; and said second porous carrier contains an immobilized, unlabeled antibody that binds to human IgG; wherein said peptide or protein comprises an epitope that is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, 90** and **141**.

In a further aspect, the invention relates to a method for detecting the presence, or measuring the concentration, of an anti-HIV-2 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of: (a) contacting said biological sample with a peptide having an epitope that is recognized by said anti-HIV-2 antibody, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody if present in said sample to bind to said epitope and form a peptide-anti-HIV-2 antibody complex; (b) contacting said formed peptide-anti-HIV-2 antibody complex with an anti-HIV-2 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody binding molecule to bind to anti-HIV-2 antibody of said formed peptide-anti-HIV-2 antibody complex and form an extended complex; and (c) determining the presence or concentration of said anti-HIV-2 antibody in said biological sample by determining the presence or concentration of said formed extended complex; wherein said epitope is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:101-102.**

In another aspect, the invention relates to a method for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of: (a) contacting said biological sample with an epitope set comprising at least one epitope that is recognized by said anti-HIV-1 antibody, wherein said epitope set consists essentially of an HIV-1 GAG p6 epitope or

epitopes, an HIV-1 gp41 terminal region epitope or epitopes, or a combination of an HIV-1 GAG p6 epitope or epitopes and an HIV-1 gp41 terminal region epitope or epitopes, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody if present in said sample to bind to epitopes in said epitope set and form an epitope-anti-HIV-1 antibody complex; (b) contacting said formed epitope-anti-HIV-1 antibody complex with an anti-HIV-1 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody binding molecule to bind to anti-HIV-1 antibody of said formed epitope-anti-HIV-1 antibody complex and form an extended complex; and (c) determining the presence or concentration of said anti-HIV-1 antibody in said biological sample by determining the presence or concentration of said formed extended complex.

In a further aspect, the invention relates to a method for detecting the presence, or measuring the concentration, of an anti-HIV-2 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of: (a) contacting said biological sample with an epitope set comprising at least one epitope that is recognized by said anti-HIV-2 antibody, wherein said epitope set consists essentially of an HIV-2 GAG p6 epitope or epitopes, an HIV-2 Env-gp36 epitope or epitopes, or a combination of an HIV-2 GAG p6 epitope or epitopes and an HIV-2 Env-gp36 epitope or epitopes, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody if present in said sample to bind to epitopes in said epitope set and form an epitope-anti-HIV-2 antibody complex; (b) contacting said formed epitope-anti-HIV-2 antibody complex with an anti-HIV-2 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody binding molecule to bind to anti-HIV-1 antibody of said formed epitope-anti-HIV-1 antibody complex and form an extended complex; and (c) determining the presence or concentration of said anti-HIV-2 antibody in said biological sample by determining the presence or concentration of said formed extended complex.

Brief Description of the Figures

Figure 1 shows a general scheme for constructing the gene fragment phage display libraries used in a preferred embodiment of the invention to obtain novel HIV epitopes.

Figure 2 shows a general scheme for panning gene fragment phage display libraries.

Figure 3 illustrates the relationship between the epitopes employed in HIV vaccines and those identified in the preferred embodiments of the present invention.

Figure 4 shows the frequency of negative samples (n=600) obtained in ELISAs employing the GAG-p6 (**SEQ ID NO:3; Panel A**) and gp41 (**SEQ ID NO:50; Panel B**) peptides.

Figure 5, Panels a-d, shows the dynamic range of serum reactivity and reproducibility of the p6 (**SEQ ID NO:3**)and gp41 (**SEQ ID NO:50 and SEQ ID NO:55)** peptide-based HIV-SELECTEST. ELISA conditions were described in **Example 9**, METHODS. A well characterized panel of nine HIV seropositive and three HIV seronegative human plasma (SeraCare BioServices) were subjected to serial two-fold dilutions starting at 1:50. Reactivity of one of the seropositive samples, PRB-204-06, with p6 (**Panel a**) and gp41 (**Panel b**) peptides is shown. Quality assurance data obtained with p6 (**Panel c**) and gp41 (**SEQ ID NO:50 and SEQ ID NO:55) (Panel d**) demonstrate the reproducibility of the new assay. In these panels, the same plasma was tested on nine dates at a 1:100 dilution. All data are represented as ratios between test specimen optical density (OD) to cut-off absorbance (CO) on the Y-axis. The Cut-off value for each peptide was determined as the mean absorbance + 5 standard deviations (SD) obtained with 1000 HIV seronegative samples. The upper and lower limits (**Panels c, d**) are the average OD/CO values $\pm$ 2SD of the plasma sample upon repeated testing, representing the 95% confidence intervals for the given control sample. Data shown represents similar results with all nine plasma samples in the control panel.

Figure 6 shows the seroreactivity of intercurrent HIV infections during HIV vaccine trials. Reactivity in the HIV-SELECTEST of sequential plasma samples obtained from intercurrent infections in the course of: (a) multiple phase I/II HIV vaccine trials conducted by the HVTN (15 vaccinees and 4 placebos) and VRC 004 (2 placebos); (b) VAX 003 phase III trial in Thailand (30 vaccinees and 35 placebos); (c) VAX 004 phase III trial in the United States and the Netherlands (53 vaccinees and 28 placebos). For each infected subject, multiple time points within 3 months of estimated infection date (panel a) or confirmed infection by PCR (depicted as day 0 on Y-axis, panels b and c) are shown vertically. Open circles (**O**) represent negative reactivity in the HIV-SELECTEST (OD/CO < 1), and filled circles (●) represent positive reactivity in the HIV-SELECTEST (OD/CO ≥ 1). Detailed reactivity data for all the samples tested are shown in **Table 27, Table 29** and **Table 30**.

Figure 7, Panels a and **b** shows comparative reactivity of early samples from HIV infections during the VAX 003 (Thailand) and VAX 004 (United States and the Netherlands) clinical trials in the HIV-SELECTEST and licensed HIV detection kits used in the VaxGen trials. Early sequential samples obtained from HIV infected vaccine trial participants in VAX 003 (30 vaccinees and 35 placebos; **Panel a**) and VAX 004 (53 vaccinees and 28 placebos; **Panel b**) were tested in the HIV-SELECTEST as described in METHODS, and by VaxGen using licensed HIV diagnostic tests. Day 0 represents the day of confirmed infection by qualitative PCR. Each dot in the figures represent the earliest bleed (post infection) on which a given infected individual scored positive by the licensed HIV diagnostic kits (X-axis) compared with the HIV-SELECTEST (Y-axis).

Description of the Preferred Embodiments

This invention relates to compositions and methods for the detection of immunodeficiency virus infection, especially immunodeficiency virus-1 (HIV-1) infection. The invention particularly concerns compositions and methods that may be used in HIV vaccine recipients whose sera may contain vaccine-generated anti-HIV-1 antibodies.

Since 1987, more than 10,000 individuals have received immunizations with human immunodeficiency virus (HIV) preventive vaccine constructs. Two large phase III trials are close to completion in the U.S and Thailand (8,000 vaccinees) and a new phase III trial is ongoing in Thailand (16,000 vaccinees). Earlier vaccine candidates were simple and usually included a single gene product, such as the viral glycoprotein gp120 or gp160. Most of the HIV-1 prophylactic vaccines currently under development are complex products containing multiple viral genes or proteins.

Unfortunately, despite such efforts, the HIV pandemic continues to take its toll globally, with more than 16,000 reported infections and 8,500 deaths occurring daily. Concerted efforts are underway to develop preventative HIV vaccines that will be both efficacious and economical. In the wake of the unsuccessful efficacy trials conducted with vaccines containing gp120 envelope alone (Francis, D.P. *et al.* (2003) "CANDIDATE HIV/AIDS VACCINES: LESSONS LEARNED FROM THE WORLD'S FIRST PHASE III EFFICACY TRIALS," Aids 17:147-56; Graham, B.S. *et al.* (2005) "LESSONS FROM FAILURE--PREPARING FOR FUTURE HIV-1 VACCINE EFFICACY TRIALS," J Infect Dis 191, 647-649), the new generation of vaccine candidates are complex products, containing multiple HIV genes or proteins, using diverse delivery systems and new adjuvants. It is anticipated that within few years several vaccine candidates will progress into large scale efficacy trials, particularly in countries with high infection rates. It is hoped that the new generation vaccines will offer at least partial protection against new infections and possibly reduce viral loads and delay disease progression in infected vaccinated individuals. In order to achieve the statistical power needed to demonstrate partial efficacy, it will be necessary to recruit thousands of volunteers into future phase III HIV vaccine trials. Many of these volunteers will react positively in licensed HIV detection tests. Hence, further improvements in HIV diagnosis are urgently required.

One of the critical determinations during ongoing trials in high-risk populations is the HIV infection status of trial participants. Intercurrent infections must be detected as soon as possible in order to stop vaccination and monitor

infected individuals for viral load, immune status, and disease progression. Treatment and public health measures depend on a timely diagnostic information. Currently, vaccine trials are using an algorithm of HIV detection that incorporates antibody or antigen-based kits, followed by Western Blots and finally, confirmatory PCR based assays. Unfortunately, many of the vaccine trial participants, irrespective of their HIV infection status, seroconvert in all licensed antibody detection kits, including the recently licensed rapid tests ((Ackers, M.L. *et al.* (2003) "HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVITY AMONG UNINFECTED HIV VACCINE RECIPIENTS," J Infect Dis 187:879-986; Pitisuttithum, P. *et al.* (2003) "SAFETY AND IMMUNOGENICITY OF COMBINATIONS OF RECOMBINANT SUBTYPE E AND B HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ENVELOPE GLYCOPROTEIN 120 VACCINES IN HEALTHY THAI ADULTS," J Infect Dis 188:219-227; Chuenchitra, T. *et al.* (2003) "LONGITUDINAL STUDY OF HUMORAL IMMUNE RESPONSES IN HIV TYPE 1 SUBTYPE CRF01_AE (E)-INFECTED THAI PATIENTS WITH DIFFERENT RATES OF DISEASE PROGRESSION," AIDS Res Hum Retroviruses 19:293-305; Schwartz, D.H. *et al.* (1995) "UTILITY OF VARIOUS COMMERCIALLY AVAILABLE HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY DIAGNOSTIC KITS FOR USE IN CONJUNCTION WITH EFFICACY TRIALS OF HIV-1 VACCINES," Clin Diagn Lab Immunol 2, 268-271). This is due to the fact that vaccine components are very similar to the diagnostic kits in composition. Therefore, recruitment of volunteers into future trials may be impeded if the informed consent forms state that volunteers are likely to seroconvert in licensed detection kits and may remain seropositive for a long time. In published surveys, it has been shown that positive HIV serodiagnosis is the most important concern for volunteers willing to participate in HIV clinical trials (Gross, M. *et al.* (1996) "INTEREST AMONG GAY/BISEXUAL MEN IN GREATER BOSTON IN PARTICIPATING IN CLINICAL TRIALS OF PREVENTIVE HIV VACCINES," J Acquir Immune Defic Syndr Hum Retrovirol 12:406-412). Thus, there is an immediate need to develop a simple and inexpensive assay that does not score uninfected vaccine recipients as positive, but provides the necessary specificity and sensitivity to detect true HIV infections in the presence of vaccine-induced antibodies.

The systems used to deliver such vaccines span the gamut from plasmid DNA to viral and bacterial vectors. Prime-boost strategies have been employed in order to optimize the cellular and humoral immune responses and reach meaningful (protective) titers and breadth of neutralizing antibodies and high frequency of cytotoxic T cells. Unfortunately, many of these constructs elicit antibodies detected by standard serologic tests for HIV-1 seroconversion. In a recent publication from the United States' Center for Disease control and Prevention, it was reported that 90% of vaccinees receiving a Canarypox construct expressing multiple HIV genes (env, gag, pol, protease, net) followed by an envelope protein boost, exhibited positive results in an enzyme immunoassay (EIA), rapid test, and Western blot (Marta-Louise Ackers *et al.* J. Infect. Dis. 2003, 187:879).

Due to increasing complexity of HIV-1 vaccine candidates, most vaccinees are expected to react positive in the licensed HIV-1 detection assays (EIA, rapid test, Western blots). There are several negative outcomes to the anticipated high prevalence of false-positives in the vaccinated individuals. These outcomes reflect the criticality of distinguishing between HIV infected recipients and individuals who have merely become seroconverted due to the administration of the vaccine:

(a) Phase III trials of prophylactic HIV-1 vaccines are typically and desirably conducted in high risk populations. As such, it is essential to diagnose break-through infections at the earliest possible times. This is necessary since infected individuals must stop vaccinations and get re-assigned to a follow-up group that are analyzed separately. Additionally, such individuals may be entitled to anti-viral therapies;

(b) Since HIV-1 infected individuals are excluded from the pool of potential blood donors, it is important to ensure both that infected individuals do not donate blood, and that individuals who have merely become seroconverted due to the administration of the

vaccine are not erroneously precluded from serving as blood donors.

(c) Since HIV-1 infected individuals face exclusions from health insurance, immigration and travel across countries, employment limitations, and preclusion from military service, it is important to be able to ensure that healthy vaccine recipients are not erroneously scored as HIV-infected individuals.

(d) Informing potential participants about the strong possibility of having reactive serologic HIV-1 tests, could deter potential volunteers from participating because of anticipated social harms. Therefore, it is important to design new testing strategies for vaccine participants that will clearly discriminate between vaccine-induced reactivity and HIV-1 infection.

The present invention addresses these concerns by providing new HIV-1 epitopes that will fulfill some, and more preferably all, of the following criteria:

(1) Do not contain important neutralizing or CTL epitopes;
(2) Are not present in HIV vaccine constructs;
(3) Are highly immunogenic in infected individuals;
(4) Are recognized by antibodies from HIV infected individuals at early times post-infection; and
(5) Are highly conserved among clades and subtypes.

Identification of the Novel Epitopes of the Present Invention

In order to identify epitopes that conform to the above criteria, it is desirable to evaluate a large pool of potential epitopes. Advantageously, a gene-fragment phage display library may be employed for this purpose. Preferably, such a library is produced using limited DNase I digestion of HIV-1 or HIV-2 DNA (**Figure 1**). Thus, target DNA, containing HIV-1 or HIV-2, is subjected to DNAse I digestion, and the digestion products are fractionated, for example using

gel electrophoresis. The termini of the fractionated samples are preferably polished using T4 polymerase (see, e.g., Costa, G.L. *et al.* (1994) "POLISHING WITH T4 OR PFU POLYMERASE INCREASES THE EFFICIENCY OF CLONING OF PCR FRAGMENTS," Nucleic Acids Res. 22(12):2423) and dephosphorylated. The treated fragments are then preferably introduced into a phagemid or phage vectors (based on filamentous phages including M13, f1, etc.) operably linked to a displayed phage protein and are then amplified to produce a phage display library, each of whose members displays an HIV peptide sequence on its surface. Alternatively, other libraries can be used including Ribosomal display, other viruses, and phages or bacterial cells or Yeast or other eukaryotic display systems for the same purpose.

Desired peptides are then identified, preferably by panning the library on immobilized serum antibodies from HIV-1-infected individual (early seroconversion) under conditions that permit the recovery of library members that bind strongly to the immobilized antibodies (**Figure 2**). Preferably, this is accomplished by capturing library members whose arrayed HIV peptide binds to an immobilized anti-HIV antibody. After discarding unbound phagemids, the bound phagemids are eluted and recovered (eluted phagemids may be amplified to enhance their recovery).

Suitable members mapping to the HIV-1 GAG-p6, RT, IN, Vif, gp120, gp41, and Nef genes, for example, can be identified in this manner. Of particular interest are members mapping to the gag, pol, Envelope and nef encoding regions of HIV. Further screenings are preferably conducted using panels of sequential sera from HIV-1 seroconvertors. **Figure 3** illustrates the relationship between the desired epitopes of the present invention, and those of vaccine candidates.

Epitopes mapping to the HIV-1 GAG-p6, gp41, Vif and Nef genes are believed to possess significant utility as diagnostic agents for the detection of anti-HIV antibodies, since, for gp41 and p6, there is >90% sequence conservation among HIV-1 clades. Moreover, gp41 and Gag-p6 peptides were recognized at high frequency (combined sensitivity of 99.1%) by serum/plasma of 1200 samples

from seropositive individuals including early seroconvertors. The specificity is currently at 98.2% for p6 and 100% for gp41 (1000 negative samples). The Gag-p6 and gp41 peptides do not contain important known neutralizing antibody or CTL epitopes and they are not present in most HIV vaccines. Identified peptides are suitable for use in HIV diagnostic kits.

In particular, the following HIV-1 peptide Gag–p6 sequences were identified as comprising desired epitopes:

C-GAG-p6 (SEQ ID NO:1)

```
SRPEPTAPPA  ESFRFGEEIT  PTPSQKQEPK  DKELYPPLAS  LRSLFGNDPS  SN
1          10          20          30          40          50  52
```

GAG-p6 (SEQ ID NO:2)

```
SRPEPTAPPE  ESFRFGEETT  TPSQKQEPID  KELYPLASLR  SLFGSDPSSQ
1          10          20          30          40          50
```

CON-GAG-p6 (SEQ ID NO:3)

```
SRPEPTAPPA  ESFRFGEEIT  PTPSQKQEPK  DKELYPPLAS  LRSLFGNDPS  SQ
1          10          20          30          40          50  52
```

CON-M-p6 (SEQ ID NO:4)

```
SRPEPTAPPA  ESFRFGEETT  PSPKQEPKDK  ELYPLTSLKS  LFGNDPLSQ
1          10          20          30          40          49
```

P6-CON-of-CON (SEQ ID NO:5)

```
SRPEPTAPPA  ESFGFGEEIT  PSPKQEPKDK  ELYPLASLKS  LFGNDPLSQ
1          10          20          30          40          49
```

CON-p6 A>E (SEQ ID NO:6)

```
SRPEPTAPPE  ESFRFGEEIT  PTPSQKQEPK  DKELYPPLAS  LRSLFGNDPS  SQ
1          10          20          30          40          50  52
```

New –GAG-p6 (SEQ ID NO:7)

```
SRPEPTAPPE  ESFRFGEEIT  PTPSQKQEPK  DKELYPLASL  RSLFGNDPSS  Q
1          10          20          30          40          50  51
```

p6-S-S (SEQ ID NO:8)

```
SRPEPTAPPA  ESFRFGEEIT  TSPSQKQEPK  DKELYPLASL  KSLFGND
1          10          20          30          40          47
```

p6-S-E (SEQ ID NO:9)

```
SRPEPTAPPA  ESFRFGEEIT  TSPSQKQEPK  DKE
1          10           20          30  33
```

p6-T-S (SEQ ID NO:10)

```
TPTPSQKQEP  KDKELYPPLA  SLRSLFGNDP  S
1          10           20          30 31
```

p6-S-Q (SEQ ID NO:11)

```
SFRFGEEITP  TPSQKQEPKD  KELYPPLASL  RSLFGNDPSS  Q
1          10           20          30 31
```

Each of these sequences was attached individually to a carboxy-terminal (**SEQ ID NO: 138**) GGGC peptide linker.

Identified GAG Epitopes

The identified GAG epitopes (**SEQ ID NOs:1-11**) differ in sequence from the sequences of previously identified GAG peptides. For example, aligning **SEQ ID NO:1** and **SEQ ID NO:2** against the HIV-1 GAG sequence (**SEQ ID NO:12**; NC_001802) yields the following comparison (sites of **SEQ ID NO:12** that are not conserved are shown in single-underline (if conserved in either **SEQ ID NO:1** or **SEQ ID NO:2**) or in double-underline (if not conserved in either **SEQ ID NO:1** or **SEQ ID NO:2**)) (**Table 1**):

Table 1

SEQ ID NO	Description	Sequence
1	Isolated Epitope	SRPEPTAPPAESFRFGEEITPTPSQKQEPKDKELYPPLASLRSLFGNDPSSN
2	Isolated Epitope	SRPEPTAPPEESFRFGEETT·TPSQKQEPIDKELYP·LASLRSLFGSDPSSQ
12	Gag	SRPEPTAPPEESFRSGVETT·TPPQKQEPIDKELYP·LTSLRSLFGNDPSSQ

Thus, both **SEQ ID NO:1** and **SEQ ID NO:2** differ in sequence from the sequence of the corresponding native HIV-1 gag gene product (**SEQ ID NO:12**). An alignment of these sequences with a series of gag consensus sequences (**Table 2**) indicates that **SEQ ID NO:1** and **SEQ ID NO:2** differ in sequence from the consensus sequences. The "$" symbol in **Table 2** indicates a stop codon.

Table 2

SEQ ID NO	Description	Sequence
12	Gag	SRPEPTAPPEESFRSGVET·T·TPPQKQEPIDKE·LYP·LTSLRSLFGNDPSSQ
1	Isolated Epitope	SRPEPTAPPAESFRFG·EI·TPTPSQKQEPKDKE·LYPPLASLRSLFGNDPSSN
2	Isolated Epitope	SRPEPTAPPEESFRFG·E··TTTPSQKQEPIDKE·LYP·LASLRSLFGSDPSSQ
13	Consensus_B (33)	SRPEPTAPPEESFRFG·EE·TTTPSQKQEPIDKE·LYP·LASLRSLFGNDPSSQ
14	Consensus_02(8)	SRPEPTAPPAESFGMG·EEIT··SSPKQEPRDKG·LYPPLASLKSLFGNDP$SQ
15	Consensus_D(8)	SRPEPTAPPAESFGFG·EEIT··PSQKQEQKDKE·LYP·LTSLKSLFGNDPLSQ
16	Consensus_F1-F2(9)	NRPEPTAPPAESFGFR·EEIT··PSPKQEQKD·EGLYPPLASLKSLFGNDP···
17	Consensus_G(5)	NRPEPTAPPAESFGFG·EEIA··PSPKQEQKEKE·LYP·LASLKSLFGSDP$SQ
18	Consensus_K (3)	SRPEPTAPPAESFGFG·EEITP··SPRQETKDKE·QGPPLTSLKSLFGNDPLSQ
19	Consensus_A-A1-A2(15)	SRPEPTAPPAEIFGMG·EEITS··PPKQEQKDRE·QNPPSVSLKSLFGNDPLSQ
20	Consensus_H (3)	SRPEPTAPPAESFGFG·EEMT··PSPKQELKDKE···PPLASLRSLFGNDPLSQ
21	Consensus_01 (11)	SRPEPTAPPAENGMGE·E·ITSLP··KQEQKDKE·HPPPLVSLKSLFGNDPLSQ
22	Consensus_c (41)	SRPEPTAPPAESFRF··EE·T·TPAPKQEPKDRE····PLTSLKSLFGSDPLSQ

The invention also relates to the following HIV 1 Gag p6 consensus epitopes shown in Table 3 below:

Table 3

SEQ ID NO	Description	Sequence				
23	Con of Cons	SRPEPTAPPA	ESFGFGEEIT	PSPKQEPKDK	ELYPLASLKS	LFGNDPLSQ
24	M.group.anc	SRPEPTAPPA	ESFGFGEEIT	PSPKQEPKDK	ELYPLASLKS	LFGSDPLSQ
25	consensus A1	SRPEPTAPPA	EIFGMGEEIT	SPPKQEQKDR	EQDPPLVSLK	SLFGNDPLSQ
26	A1.anc	SRPEPTAPPA	ENFGMGEEMI	SSPKQEQKDR	EQYPPLVSLK	SLFGNDPLSQ
27	consensus A2	SRTEPTAPPA	ENLRMGEEIT	SSLKQELKTR	EPYNPAISLK	SLFGNDPLSQ
28	consensus B	SRPEPTAPPE	ESFRFGEETT	TPSQKQEPID	KELYPLASLR	SLFGNDPSSQ
29	B.anc	SRPEPTAPPE	ESFRFGEETT	TPSQKQEPID	KELYPLASLK	SLFGNDPSSQ
30	consensus C	NRPEPTAPPA	ESFRFEETTP	APKQEPKDRE	PLTSLKSLFG	SDPLSQ
31	C.anc	SRPEPTAPPA	ESFRFEETTP	APKQEPKDRE	PLTSLKSLFG	SDPLSQ
32	consensus D	SRPEPTAPPA	ESFGFGEEIT	PSQKQEQKDK	ELYPLTSLKS	LFGNDPLSQ
33	consensus F1	SRPEPTAPPA	ESFGFREEIT	PSPKQEQKDE	GLYPPLASLK	SLFGNDP
34	consensus G	NRPEPTAPPA	ESFGFGEEIA	PSPKQEQKEK	ELYPLASLKS	LFGSDP
35	consensus H	SRPEPTAPPA	ESFGFGEEMT	PSPKQELKDK	EPPLASLRSL	FGNDPLSQ
36	consensus K	SRPEPTAPPA	ESFGFGEEIT	PSPRQETKDK	EQGPPLTSLK	SLFGNDPLSQ
37	consensus_01_AE	SRPEPTAPPA	ENWGMGEEIT	SLPKQEQKDK	EHPPPLVSLK	SLFGNDPLSQ
38	consensus_02_AG	SRPEPTAPPA	ESFGMGEEIT	SSPKQEPRDK	GLYPPLTSLK	SLFGNDP
39	consensus_03_AB	SRPEPSAPPA	ENFGMGEEIT	PSLKQEQKDR	EQHPPSISLK	SLFGNDPLSQ
40	consensus_04_CPX	SRPEPTAPPA	ESLEMKEETT	SSPKQEPRDK	ELYPLTSLKS	LFGSDPLSQ
41	consensus_06_CPX	NRPEPTAPPA	ESFGFGEETA	PSPKQEPKEK	ELYPLASLKS	LFGNDP
42	consensus_07_BC	SRPEPTAPPE	ESFRFGEETT	TPSQKQEPID	KELYPLTSLK	SLFGNDPSSQ
43	consensus_08_BC	SRPEPTAPPA	ESFRFEETTP	APKQEPKDRE	PLTSLRSLFG	SDPLSQ
44	consensus_10_CD	SRPEPTAPPA	ESFGFGEEIT	PSQKQEQKDK	ELHPLASLKS	LFGNDPLSQ
45	consensus_11_CPX	SRPEPTAPPA	ESFGFGEEIA	PSPKQEPKEK	ELYPLTSLKS	LFGSDPLSQ
46	consensus_12_BF	NRPEPTAPPA	ESFGFGEEIT	PSPKQEQKDE	GLYPPLASLK	SLFGNDP
47	consensus_14_BG	NRPEPTAPPA	ESFGFGEEIA	PSPKQEPKEK	EIYPLASLKS	LFGSDP$SQ

A blast search of **SEQ ID NO:1** failed to identify any identical gene sequences. A blast search of **SEQ ID NO:2** identified three sequences that were 100% identical to **SEQ ID NO:2** (Sequence **AAC28445** (Fang, H. *et al.*, (1995) J. Virol. 69(1):75-81; Sequence **P12493** (Buckler, C.E. *et al.* Direct Submission); Sequence **AAB04036** (Willey, R.L. *et al.* (1986) Proc. Natl. Acad. Sci. U.S.A. 83 (14), 5038-5042).

U.S. Patent No. 6,458,527 relates to an immunoassay to detect the presence of a human immunodeficiency virus using a GAG antigen that comprises at least seven contiguous amino acids from a GAG open reading frame that includes **SEQ ID NO:48**:

SRPEPTAPPE	ESFRFGEEKT	TTPSQKQEPI	DKELYPLT S	LRSLFGNDPS	SN
2149	2179	2209	2239	2269	2299

A comparison of this sequence to **SEQ ID NO:1 and SEQ ID NO:2** indicates that these sequences share more than seven contiguous residues with **SEQ ID NO:48**) (**Table 4**):

Table 4

SEQ ID NO	Description	Sequence
48	GAG	SRPEPTAPPEESFRFGEEKTTTPSQKQEPIDKELYPLT·SLRSLFGNDPSSN
1	Isolated Epitope	SRPEPTAPPAESFRFGEEITPTPSQKQEPKDKELYPPLASLRSLFGNDPSSN
2	Isolated Epitope	SRPEPTAPPEESFRFGEET··TPSQKQEPIDKELYPL·ASLRSLFGSDPSSQEF

The identified Gag epitope sequences (**SEQ ID NO:1** or **SEQ ID NO:2**), or any of **SEQ ID NOS:3-47** are useful as diagnostic agents in accordance with the principles of the present invention. **SEQ ID NOs:1-7** are preferred as a gag epitope sequences, and **SEQ ID NO:3** is particularly preferred as a gag epitope sequence.

Identified gp41 Epitopes

HIV-1 Env-gp41 (SEQ ID NO:49):

LIAARTVELL	GHSSLKGLRL	GWEGLKYLWN	LLLYWGRELK	ISAINLVDTI	AIAVAGWTDR
1 10	20	30	40	50	60

VIEIGQRIGR AILHIPRRIR QGLERALL
70 80 88

CON-ENV-2-gp41 (SEQ ID NO:50):

```
LIAARIVELL  GHSSLKGLRR  GWEALKYLWN  LLQYWGQELK  NSAISL
1           10          20          30          40      46
```

ENV-gp41 (SEQ ID NO:51):

```
LIVTRIVELL  GRRGWEALKY  WWNLLNYWSQ  ELKNSAVNL
1           10          20          30          39
```

Env2-gp41-A-Q (SEQ ID NO:52)

```
ARIVELLGHS  SLKGLRRGWE  ALKYLWNLLQ  YWGQ
1           10          20          30  34
```

Env3-gp41-seq (SEQ ID NO:53)

```
CRAILNIPRR  IRQGLERALL
1           10          20
```

Env4-gp41 seq (SEQ ID NO:54)

```
AVAEGTDRVI  EVVQRV
1           10      16
```

Env34-gp41 seq (SEQ ID NO:55)

```
AVAEGTDRVI  EVVQRVCRAI  LNIPRRIRQG  FERALL
1           10          20          30      36
```

The identified gp41 epitopes (**SEQ ID NO:50** and **SEQ ID NO:51**) differ in sequence from the sequences of previously identified gp41 peptides. Aligning **SEQ ID NO:50** and **SEQ ID NO:51** against the HIV-1 gp41 sequence (**SEQ ID NO:56**; NC_001802) yields the following comparison (sites of **SEQ ID NO:56** that are not conserved are shown in single-underline (if conserved in either **SEQ ID NO:50** or **SEQ ID NO:51**) or in double-underline (if not conserved in either **SEQ ID NO:50** or **SEQ ID NO:51**)) (**Table 5**):

Table 5

SEQ ID NO	Description	Sequence
50	Isolated Epitope	LIAARIVELLGHSSLKGLRRGWEALKYLWNLLQYWGQELKNSAISL
51	Isolated Epitope	LIVTRIVELLG·····RRGWEALKYWWNLLQYWSQELKNSAVNL
56	Gp41	LIVTRIVELLG·····RRGWEALKYWWNLLQYWSQELKNSAVSL

Thus, both **SEQ ID NO:50** and **SEQ ID NO:51** differ in sequence from the sequence of the corresponding native HIV-1 gp41 gene product (**SEQ ID NO:56**). An alignment of these sequences with a series of gp41 consensus sequences (**Table 6**) indicates that **SEQ ID NO:50** and **SEQ ID NO:51** differ in sequence from the consensus sequences.

Table 6

SEQ ID NO	Description	Sequence
56	GP41	LIVTRIVELLG·····RRGWEALKYWWNLLQYWSQELKNSAVSL
50	Isolated Epitope	LIAARIVELLGHSSLKGLRRGWEALKYLWNLLQYWGQELKNSAISL
51	Isolated Epitope	LIVTRIVELLG·····RRGWEALKYWWNLLQYWSQELKNSAVNL
57	Consensus_B (128)	LIVTRIVELLG·····RRGWEALKYWWNLLQYWSQELKNSAVNL
58	Consensus_D (17)	LIAARIVELLG·····RRGWEALKYLWNLLQYWIQELKNSAISL
59	Consensus_F1-F2 (10)	LIAARTVDRGL·····KRGWEALKYLWNLTQYWGQELKNSAISL
60	Consensus_H (3)	LIVVRTVELLG·····RRGREALKYLWNLLQYWGQELKNSAINL
61	Consensus_A-A1-A2 (24)	LIAARTVELLGHSSLKGLRLGWEGLKYLWNLLLYWGRELKISAINL
62	Consensus_C (50)	LIAARAVELLGRSSLRGLQRGWEALKYLGSLVQYWGLELKKSAISL
63	Consensus_G (9)	LIAARTVELLGRSSLKGLRLGWEGLKYLWNLLLYWGQELKNSAINL
64	Consensus_01 (32)	LIAARTVELLGHSSLKGLRRGWEGLKYLGNLLLYWGQELKISAISL
65	Consensus_02 (16)	LIAARTVELLGHSSLKGLRLGWEALKYLGNLLSYWGQELKNSAINL

A blast search of **SEQ ID NO:50** failed to identify any identical gene sequences. A blast search of **SEQ ID NO:51** identified two sequences that were 100% identical to **SEQ ID NO:51** (Sequence **AAC28452** (Fang, H. *et al.* (1995) J. Virol. 69 (1), 75-81; Sequence **P04582** (Ratner, L. *et al.* (1985) Nature 313:277-284 (1985). Of the sequences that differed from **SEQ ID NO:51** by one amino acid residue, a substitution of L38→N38 was common (see, e.g., Sequence **CAD10927** (Zheng, N.N. *et al.*, Direct Submission). **SEQ ID NOs:50, 53 and 55** are particularly preferred as gp41 epitope sequences.

The invention also relates to the following HIV 1 gp41 consensus epitopes shown in Table 7 below:

Table 7

SEQ ID No.	Description	Sequence
66	CON_OF_CONS	LIAARTVELLGRRGWEALKYLWNLLQYWGQELKNSAISLLDTTAIAVAEGT DRVIEVVQRVCRAILNIPRRIRQGFERALL
67	Mgroup.anc	LIAARTVELLGRRGWEALKYLWNLLQYWGQELKNSAISLLDTTAIAVAEGT DRVIEVVQRACRAILHIPRRIRQGFERALL
68	CONSENSUS_A1	LIAARTVELLGHSSLKGLRLGWEGLKYLWNLLLYWGRELKISAINLVDTIA IAVAGW TDRVIEIGQRIGRAILHIPRRIRQGLERALL
69	A1.anc	LIAARTVELLGRSSLKGLRLGWEGLKYLWNLLLYWGRELKISAINLLDTIA IAVAGWTDRVIEIGQRICRAILNIPRRIRQGLERALL
70	CONSENSUS_A2	LIAARTVELLGHSSLKGLRLGWEGLKYLWNLLLYWGRELKNSAISLLDTIA VAVAEWTDRVIEIGQRACRAILNIPRRIRQGFERALL
71	CONSENSUS_B	LIVTRIVELLGRRGWEVLKYWWNLLQYWSQELKNSAVSLLNATAIAVAEGT DRVIEVVQRACRAILHIPRRIRQGLERALL
72	B.anc	LIVARIVELLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNATAIAVAEGT DRVIEVVQRACRAILHIPRRIRQGLERALL
73	CONSENSUS_C	LIAARAVELLGRSSLRGLQRGWEALKYLGSLVQYWGLELKKSAISLLDTIA IAVAEGTDRIIELIQRICRAIRNIPRRIRQGFEAALQ
74	C.anc	LIAARAVELLGRSSLRGLQRGWEALKYLGSLVQYWGLELKKSAISLLDTIA IAVAEGTDRIIELIQRICRAIRNIPRRIRQGFEAALL
75	CONSENSUS_D	LIAARIVELLGRRGWEALKYLWNLLQYWIQELKNSAISLFDTTAIAVAEGT

		DRVIEIVQRACRAILNIPTRIRQGLERALL
76	CONSENSUS_F1	LIAARIVDRGLRRGWEALKYLGNLTQYWSQELKNSAISLLNTTAIVVAEGT DRVIEALQRAGRAVLNIPRRIRQGLERALL
77	CONSENSUS_F2	LIAARTVDMGLKRGWEALKYLWNLPQYWGQELKNSAISLLDTTAIAVAEGT DRIIEVLQRAGRAVLHIPRRIRQGFERALL
78	CONSENSUS_G	LIAARTVELLGRSSLKGLRLGWEGLKYLWNLLLYWGQELKNSAINLLDTIA IAVANWTDRVIEVAQRACRAILNIPRRIRQGLERALL
79	CONSENSUS_H	LIVVRTVELLGRRGREALKYLWNLLQYWGQELKNSAINLLNTTAIAVAEGT DRIIEIVQRAWRAILHIPRRIRQGFERTLL
80	CONSENSUS_01_AE	LIAARTVELLGHSSLKGLRRGWEGLKYLGNLLLYWGQELKISAISLLDATA IAVAGWTDRVIEVAQGAWRAILHIPRRIRQGLERALL
81	CONSENSUS_02_AG	LIAARTVELLGHSSLKGLRLGWEALKYLGNLLSYWGQELKNSAINLLDTIA IAVANWTDRVIEIGQRAGRAILNIPRRIRQGLERALL
82	CONSENSUS_03_AB	LIAARIVELLGRRGWEALKYWWNLLQYWIQELKSSAINLIDTIAIAVAGWT DRVIEIGQRFCRAIRNIPRRIRQGAEKALQ
83	CONSENSUS_04_CPX	LIVARTVELLGIRGWEALKYLWNLLLYWGQELRNSAINLLDTTAIAVAEGT DRIIEAVQRACRAIRNIPRRIRQGLERALL
84	CONSENSUS_06_CPX	LIAARTVETLGHRGWEILKYLGNLVCYWGQELKNSAISLLDTTAIAVANWT DRVIEVVQRVFRAFLNIPRRIRQGFERALL
85	CONSENSUS_08_BC	LTARGVELLGRNSLRGLQRGWEALKYLGSLVQYWGLELKKSTISLVDTIAI AVAEGTDRIINIVQGICRAIHNIPRRIRQGFEAALQ
86	CONSENSUS_10_CD	LIATRIVELLGRRGWEAIKYLWNLLQYWIQELKNSAISLLDTTAIAVAEGT DRAIEIVQRAVRAVLNIPTRIRQGLERALL
87	CONSENSUS_11_CPX	LIAARIVETLGRRGWEILKYLGNLAQYWGQELKNSAISLLNATAIAVAEGT DRIIEVVHRVLRAILHIPRRIRQGFERALL
88	CONSENSUS_12_BF	LIVTRIVELLGRRGWEVLKYWWNLLQYWSQELKNSAISLLNTTAIVVAEGT DRVIEALQRVGRAILNIPRRIRQGLERALL
89	CONSENSUS_14_BG	LIAARTVELLGRSSLKGLRLGWEGLKYLWNLLLYWGRELKNSAINLLDTVA IAVANWTDRAIEVVQRVGRAVLNIPVRIRQGLERALL

The identified HIV-1 gp41 epitope sequences (**SEQ ID NO:50** or **SEQ ID NO:55**), or any of **SEQ ID NOS:49-89** are useful as diagnostic agents in accordance with the principles of the present invention.

As one embodiment of the invention, diagnostic assays are contemplated wherein an HIV-1 epitope sets are employed wherein the peptide epitope sets consist essentially of HIV-1 gp41 terminal region epitopes or HIV-1 GAG p6 epitopes or a combination of gp41 and GAG p6 epitopes. As used herein, "HIV-1 gp41 terminal region epitopes" refers to epitopes contained on amino acids 784-871 of Consensus aligned seq in Los Alamos database, or mutants or derivatives thereof. As used herein HIV-1 GAG-p6 epitopes refers to to epitopes on amino acids 452-502 of Consensus aligned sequence in Los Alamos database, or mutants or derivatives thereof. As used herein, an epitope set will consist essentially of gp41 and GAG p6 epitopes when the peptide epitope set does not contain other epitopes that show significant reactivity (i.e. preferably less than 130% of background level, more preferably less than 120% of background level, and most preferably less than 110% of background level) with anti-HIV 1 antibodies.

Identified Nef Epitope

NEF Peptide (SEQ ID NO:90):

```
GLIHSQRRQD  ILDLWIYHTQ  GYFPDWQNYT  PGPGVRYPL
1         10          20          30         39
```

The identified Nef epitope (**SEQ ID NO:90**) is identical in sequence with the HIV-1 Nef sequence (NC_001802) (**SEQ ID NO:91**). An alignment of these sequences with a series of Nef consensus sequences (**Table 8**) indicates that **SEQ ID NO:90** is highly conserved.

Table 8

SEQ ID NO	Description	Sequence
91	nef	GLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPL
90	Isolated Epitope	GLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPL
92	Consensus_B (266)	GLIYSQKRQDILDLWVYHTQGYFPDWQNYTPGPGIRYPL
93	Consensus_D (4)	GLIWSQKRQEILDLWVYHTQGFFPDWQNYTPGPGIRYPL
94	Consensus_A-A1-A2 (14)	GLIYSKKRQEILDLWVYHTQGYFPDWQNYTPGPGIRYPL
95	Consensus_C (44)	GLIYSKKRQEILDLWVYHTQGYFPDWQNYTPGPGVRYPL
96	Consensus_F1-F2 (7)	GLIYSKKRQDILDLWVYHTQGYFPDWQNYTPGPGIRYPL
97	Consensus_01 (31)	GLIYSKKRQEILDLWVYNTQGFFPDWQNYTPGPGIRYPL
98	Consensus_02 (12)	GLIYSKKRQEILDLWVYHTQGFFPDWQNYTPGPGTRFPL
99	Consensus_G (7)	GLIYSKKRQEILDLWVYNTQGFFPDWQNYTPGPGTRFPL
100	Consensus_H (5)	GLIYSKKRQEILDLWVYNTQGYFPDWQNYTPGPGERYPL

The identified nef epitope sequence (**SEQ ID NO:90**), or any of **SEQ ID NOS:92-100** are useful as diagnostic agents in accordance with the principles of the present invention.

Identified HIV-2 epitopes

As one aspect of the invention, it is contemplated that peptides comprising epitopes that are contained on HIV-2 Env-gp36 (e.g. amino acids 817-927 of MAC.US.-.239_M33262 ENV aligned seq in Los Alamos database, or mutants or derivatives thereof) or epitopes that are contained on HIV-2 GAG-p6 (amino acids 461-555 of MAC.US.-.239_M33262 GAGPRO aligned seq in Los Alamos database, or mutants or derivatives thereof) will be useful in the diagnosis of HIV-2 infection. In particular, it is contemplated as an aspect of the invention that a combination of at least one epitope contained on HIV-2 gp36 and at least one epitope contained on HIV-2 GAG-p6 will be useful in the diagnosis of

breakthrough HIV-2 infections in individuals that have been vaccinated against the HIV-1 or HIV-2 virus, or viral genes or gene products of these viruses.

HIV-2 GAG-p6 (SEQ ID NO:101)

```
APQGLIPTAP  PMNPAFGMTP  QGAIPSAPPA  DPAADLLEKY  LQQGRKQREQ  RERPYKEVTE
1          10          20          30          40          50          60

DLLHLEQGET  PRREATEDLL  HLNSLFGKDQ
           70          80          90
```

HIV-2 Env-gp36 (SEQ ID NO:102)

```
FLIRLLIRLL  IGLYNICRTL  ISKSFQTLQP  ISQGLQRALT  AIRDWLRPGA  AYLQYGCEWI
1          10          20          30          40          50          60

QEALQAFARA  TRETLTSVWR  NFCGTMGQIG  RGILAIPRRI  RQGAELALL
           70          80          90          100         109
```

The invention also relates to HIV-2 GAG p6 and HIV-2 Env-gp36 epitopes shown below in Table 9 and Table 10, respectively.

Table 9

SEQ ID NO	Description	Sequence
103	MAC.US.-.239 M33262	VHQGLMPTAPPEDPAVDLLKNYMQLGKQQREKQRESREKPYKEVTEDLLHLN SLFGGDQ
104	H2A.CI.88.UC2 U38293	APQGLIPTAPPADPAADLLEKYLQQGRKQREQRERPYKEVTEDLLHLEQGET PRRE-ATEDLLHLNSLFGKDQ
105	MAC.US.-.MM142 M16403	VHQGLTPTAPPEEPAVDLLKNYMHLGKQQRESRGKPYKEVTEDLLHLNSLFG GDQ
106	MNE.US.-.MNE027 U79412	MHQGLTPTAPPEDPAVDLLKNYMQLGKQQRESKRKPYKEVAEDLLHLNSLFG EDQ
107	SMM.US.-.H9 M80194	MPQGLTPTAPPEDPAVDLLKNYMKVGRRQRENRERPYKEVTEDLLHLNSLFG EDQ
108	H2B.CI.-.EHO U27200	APQGIVPSAPPMNPAFGMTPQGAIPSAPPADPAEEMLKNYMQLGKKQKENRE RPYKEVTEDLLHLNSLFGEDQ
109	H2B.CI.88.UC1 L07625	VPQGVTPSAPPMDPAEGMTPRGATPSAPPADPAVEMLKSYMKMGRQQRESRE RPYKEVTEDLLHLNSLFGEDQ
110	H2B.GH.86.D205 X61240	VPQGVTPSAPPMNPAEGMTPRGATPSAPPADPAVEMLKSYMQMGRQQRESRE RPYKEVTEDLLHLNSLFGEDQ
111	H2B.JP.01.KR020 AB100245	APQGILPSAPPMNPAENMTPQGAMPSAPPADPAVEMLKDYMQLGRKQKGGRE KPYKEVTEDLLHLNSLFGEDQ
112	H2G.CI.-.ABT96 AF208027	VPQGLTPSAPPMDPAVDLLKNYMQLGRKQKEQRNKPYKEVTEXLLHLSSLFG DDQ
113	H2U.FR.96.12034 AY530889	VPQGLTPTAPPAEPAVDLLTPTAPPADPAVDLLKSYMQQGKKQKENRERPYK EVTEDLLHLNSLFGNDQ
114	H2AB.CI.-.7312A L36874	VPQEIVPSAPPMNTAEGKTHQGAIPSAPPADPAVEMLKSYMQLGKQQREKQG RPYKEVTEDLLHLNSLFGEDQ
115	SMM.SL.92.SL92B AF334679	TTSLTPSAPPDPAARIVKEYLEKAQREKTRRSRPYKEVTEDLLHLNSLFGED Q

Table 10

SEQ ID NO	Description	Sequence
116	MAC.US.-.239_M33262	FLIRQLIRLLTWLFSNCRTLLSRVYQILQPILQRLSATLQRIREVLRTELTYLQYGW SYFHEAVQAVWRSATETLAGAWGDLWETLRRGGRWILAIPRRIRQGLELTLL
117	H2A.-.-.CBL21_U0535	FLIRLLIRLLIGLYNICRTLISKSFQTLQPISQGLQRALTAIRDWLRPGAAYLQYGC EWIQEALQAFARATRETLTSVWRNFCGTMGQIGRGILAIPRRIRQGAELALL

	0	
118	H2A.CI.88.UC2_U38293	LLIHLLTRLLTGLYSICRDLLSANSPTRRLISQNLTAIRDWLRLKAAYLQYGCEWIQEAFQAIARTARETLAGAWRGLCKAVQRIGRGILAVPRRIRQGAEIALL
119	H2A.DE.-.BEN_M30502	FLIHLLTRLLIGLYNICRDLLSKNSPTRRLISQSLTAIRDWLRLKAAQLQYGCEWIQEAFQAFARTTRETLAGAWGWLWEAARRIGRGILAVPRRIRQGAELALL
120	H2A.GH.-.GH1_M30895	FLIHLLTRLLTGLYKICRDLLSTNSPTHRLISQNLTAIRDWLRLKAAYLQYGGEWIQEAFQAFAKTTRETLASAWGGLCAAVQRVGRGILAVPRRIRQGAEIALL
121	H2A.GM.87.D194_J04542	FLIHLLTRLLTGLYNSCRGLLSKNSPTRRLISQSLTAIRDWLRLKAAYLQYGCEWIQEAFRAFARTARETIAGAWRGLCEAAQRIGRGILAVPRRIRQGAEIALL
122	II2A.GM.90.CBL24_U05353	FLIRLLIRLLIGLYNICRDLLSRSSLILQPILQSLQRALTAIRDWLRLEAAYLQYGCEWIQEALQALTRATRETLAGAWRNLWGALQRIGRGILAVPRRIRQGAELALL
123	H2A.GW.-.CAM3_U05355	FLIRLLIRLLTRLYNSCRDLLSRSFLTLQPIFQNLRDWLRLRTAFLQYGRQWIQEAFQAFARATRETLTSACRGLWRTLDNFGRGILSIPRRIRQGAEIALL
124	H2A.SN.-.ST_M31113	FLIRQLIRLLNRLYNICRDLLSRSFQTLQLISQSLRRALTAVRDWLRFNTAYLQYGGEWIQEAFRAFARATGETLTNAWRGFWGTLGQIGRGILAVPRRIRQGAEIALL
125	H2A.SN.85.ROD_M15390	FLIRQLIRLLTRLYSICRDLLSRSFLTLQLIYQNLRDWLRLRTAFLQYGCEWIQEAFQAAARATRETLAGACRGLWRVLERIGRGILAVPRRIRQGAEIALL
126	H2A.GM.-.ISY_J04498	FLIRLLIRLLTRLYNSCRDLLSRLYLILQPLRDWLRLKAAYLQYGCEWIQEAFQALARVTRETLTSAGRSLWGALGRIGRGILAVPRRIRQGAEIALL
127	H2B.CI.-.EHO_U27200	FPIRQLRDLLIWLYSGCRTLLSKTFQTLQPVLQPLRLPPAYLRYGISWFQEAIQAAARRAAGETLASAARTSWGVLRRAAGEIIAIPRRIRQGAELALL
128	H2B.GH.86.D205_X16109	FLLRQLRNLLIWLYNGCRTLLLKTFQILHQISTNLQPLRLPVAYLQYGISWFQEALRAAARATGETLASAGETLWEALRRAARAIIAIPRRIRQGLELTLL
129	H2G.CI.-.ABT96_AF208027	FLXRQLGNLLTWLYSNCRALLSRIXQTLQPLFQRISRTLQAIREHLRLEAAYFSYGFRWLQEACTAATRAAQETLTSTWRALWKTLGRVGRGILAIPRRIRQGLELTLL
130	H2U.FR.96.12034_AY530889	FLIHQLIRLLTWLYSSCRDLLSRICQSLQPLFQSIRERLHLEIAYLQYGWQYFKEAFQAFGKAARETLSRTGRELWETLGRVGRWLRAIPRRIRQGFELALL
131	H2AB.CI.-.7312A_L36874	FLIRQLRNLLIWLYDGCRTLLLKTFQTLQPALQPLRLLFAYLQYGIGWFQEAVQAAAGATGETLASTGRTLWEALRRTARGIIAVPRRIRQGLELALL
132	MAC.US.-.BR5_AY290716	FLIRQLIRLLTWLFSNCRTLLSRVYQILQPIFQRLSATLQRIREVLRTELTYLQYGWSYFHEAVQAVWRSATETLAGAWGDLWETLRRGGRW
133	MAC.US.-.BR5_AY290710	FLIRQLIRLLTWLFSNCRTLLSRVYQILQPMFQGLSATLQRIREVLRTELTYLQYGWSYFHEAVQAVWRAATETLAGAWGDLWETLRRGGRW
134	MAC.US.-.BR5_AY290709	FLIRQLIRLLTWLFSNCRTLLSRVYQILQPIFQGLSATLQRIREVLRTELTYLQYGWSYFHEAVQAVWRAATETLAGAWGDLWETLRRGGRW
135	MNE.US.-.MNE027_U79412	FLIRQLIRLLTWLFSNCRTLLSRAYQILQPIFQRFSTTLQRVREVLRTELTYLQYGWSYFQEAVQVAWRSATETLAGAWGDLWETLGRVGRWILAIPRRIRQELELTLL
136	SMM.SL.92.SL92B_AF334679	FLIRQLIRILTWLYNNLTRLASRAYQNLQQLCQRLSEISQPIRELVRREAGYIRYGWNYFIEACQEAWRSAQEAIVGAWGLIWETLGRVGRGIAAIPRRIRQGLELMLN
137	SMM.US.-.PT583_AY221512	FLIRQLIRLLTWLFSSCRDWLLRIYQILQPVLQGLSRTLQRVREVIRIEITYLQYGWSYFQEAAQAWWKFARETLASAWRDIWETLGRVGRGILAIPRRVRQGLELALL

The identified HIV-2 GAG-p6 epitope sequence (**SEQ ID NO:101**), or any of **SEQ ID NOS:103-115** are useful as diagnostic agents in accordance with the principles of the present invention. Likewise, the identified HIV-2 Env-gp36 epitope sequence (**SEQ ID NO:102**), or any of **SEQ ID NOS:116-137** are useful as diagnostic agents in accordance with the principles of the present invention.

As one embodiment of the invention, diagnostic assays are contemplated wherein HIV-2 epitope sets are employed, wherein the epitope sets consist essentially of HIV-2 GAG-p6 epitopes or HIV-2 Env-gp36 epitopes. As used herein, an epitope set will consist essentially of HIV-2 GAG-p6 epitopes and HIV-2 Env-gp36 epitopes when the peptide epitope set does not contain other epitopes that show significant reactivity (i.e. preferably less than 130% of background level,

more preferably less than 120% of background level, and most preferably less than 110% of background level) with anti-HIV 2 antibodies.

Peptide molecules containing the epitopes of the invention may be prepared using virtually any art-known technique for the preparation of peptides. For example, the peptides may be prepared using conventional step-wise solution or solid phase peptide syntheses, or recombinant DNA techniques or proteolysis or modifications of purified viral proteins/peptides or recombinant proteins. Peptides may be prepared using conventional step-wise solution or solid phase synthesis (see, e.g., Merrifield, R B. (1969) "SOLID-PHASE PEPTIDE SYNTHESIS," Adv. Enzymol. Relat Areas Mol. Biol. 32:221-296; Fairwell, T. et al. (1987) "HUMAN PLASMA APOLIPOPROTEIN C-II: TOTAL SOLID-PHASE SYNTHESIS AND CHEMICAL AND BIOLOGICAL CHARACTERIZATION," Proc. Natl. Acad. Sci. U. S. A 84:4796-4800; Kent, S.B.H. "CHEMICAL SYNTHESIS OF PEPTIDES AND PROTEINS," (1988) Ann. Rev. Biochem. 57, 957-984, CHEMICAL APPROACHES TO THE SYNTHESIS OF PEPTIDES AND PROTEINS, Williams et al., Eds., 1997, CRC Press, Boca Raton Fla., and references cited therein; SOLID PHASE PEPTIDE SYNTHESIS: A PRACTICAL APPROACH, Atherton & Sheppard, Eds., 1989, IRL Press, Oxford, England, and references cited therein).

Alternatively, such peptides of the invention may be prepared by way of segment condensation, as described, for example, in Schnölzer, M. *et al,*. "CONSTRUCTING PROTEINS BY DOVETAILING UNPROTECTED SYNTHETIC PEPTIDES: BACKBONE-ENGINEERED HIV PROTEASE," Science. 1992 Apr 10;256(5054):221-5; Schnölzer, M., "IN SITU NEUTRALIZATION IN BOC-CHEMISTRY SOLID PHASE PEPTIDE SYNTHESIS. RAPID, HIGH YIELD ASSEMBLY OF DIFFICULT SEQUENCES," *Int J Pept Protein Res.* 1992 Sep-Oct;40(3-4):180-193; Rose *et al.*, "STEPWISE SOLID-PHASE SYNTHESIS OF POLYAMIDES AS LINKERS," J. Am. Chem. Soc. 1999 August 4, 121:7034-7038. Methods for preparing peptides are disclosed in United States Patent Nos. 6,004,925 and 6,429,289.

Assays for the detection of HIV-1 and HIV-2

The present invention is directed in part to the use of novel epitopes in diagnostic assays for the detection of HIV-1 or HIV-2. In a preferred embodiment, such assays of HIV will comprise enzyme immunosorbent assays (**EIAs**) that employ one or more of the above-described gp41, GAG and/or nef peptides, or fragments or variants thereof, or one or more of the above described HIV-2 GAG or Env-gp36 peptides, or fragments or variants thereof. In a preferred embodiment, 1, 2 or 3 such peptides are employed in such assays. The selected peptides, alone or in combination, can be used to differentiate between vaccine directed antibodies and breakthrough infection generated antibodies to assess the efficacy of vaccine clinical trials, or to monitor potential infections.

Fragments or variants of the peptides preferably comprise at least 10, 20 or 30 contiguous amino acids of the peptides and are at least 70%, preferably at least 75%, 80%, or 85%, more preferably at least 90%, and most preferably at least 95% homologous to the indicated peptides.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence (i.e. homology), also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., 1990, *Comp. App. Biosci.*6:237-245. In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=l, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N-

and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total residues of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The present invention concerns the binding of peptide eptitopes and antibodies. As used herein, an "epitope" is a 2- or 3-dimensional region of an antigen that is recognized by and that specifically binds to an antibody. As used herein, an epitope and an antibody are said to be "specific" for one another, or to "recognize" one another, or to "bind" to one another if they are capable of immunospecific binding to one another.

Any of a wide variety of assay formats may be used in accordance with the methods of the present invention. Such formats may be heterogeneous or homogeneous, sequential or simultaneous, competitive or noncompetitive. U.S. Patent Nos. 5,563,036; 5,627,080; 5,633,141; 5,679,525; 5,691,147; 5,698,411; 5,747,352; 5,811,526; 5,851,778; and 5,976,822 illustrate several different assay formats and applications. Such assays can be formatted to be quantitative, to measure the concentration or amount of an anti-HIV antibody, or they may be formatted to be qualitative, to measure the presence or absence of an anti-HIV antibody. Additional descriptions of immunoassays that may be adapted for use in accordance with the principles of the present invention are available in the scientific literature (Gnann, J.W. Jr *et al.* "CUSTOM-DESIGNED SYNTHETIC PEPTIDE IMMUNOASSAYS FOR DISTINGUISHING HIV TYPE 1 AND TYPE 2 INFECTIONS," Methods Enzymol. 1989;178:693-714; Dopel, S.H. *et al.* "COMPARISON OF FOUR ANTI-HIV SCREENING ASSAYS WHICH BELONG TO DIFFERENT TEST GENERATIONS," Eur J Clin Chem Clin Biochem. 1991 May;29(5):331-7; Manocha, M. *et al.* "COMPARING MODIFIED AND PLAIN PEPTIDE LINKED ENZYME IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) AND TYPE-2 (HIV-2) ANTIBODIES," Immunol Lett. 2003 Feb 3;85(3):275-8); Brattegaard, K. *et al.* "INSENSITIVITY OF A SYNTHETIC PEPTIDE-BASED TEST (PEPTI-LAV 1-2) FOR THE DIAGNOSIS OF HIV INFECTION IN AFRICAN CHILDREN," AIDS. 1995 Jun;9(6):656-7; Beristain, C.N. *et al.* "EVALUATION OF A DIPSTICK METHOD FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION," J Clin Lab Anal. 1995;9(6):347-50; Modrow, S. *et al.* "CARRIER BOUND SYNTHETIC OLIGOPEPTIDES IN ELISA TEST SYSTEMS FOR DISTINCTION BETWEEN HIV-1 AND HIV-2 INFECTION," J Acquir

Immune Defic Syndr. 1989;2(2):141-8; Gueye-Ndiaye, A. *et al.* "COST-EFFECTIVE DIAGNOSIS OF HIV-1 AND HIV-2 BY RECOMBINANT-EXPRESSED ENV PEPTIDE (566/996) DOT-BLOT ANALYSIS," AIDS. 1993 Apr;7(4):475-81; Sabatier, J.M. *et al.* "USE OF SYNTHETIC PEPTIDES FOR THE DETECTION OF ANTIBODIES AGAINST THE NEF REGULATING PROTEIN IN SERA OF HIV-INFECTED PATIENTS," AIDS. 1989 Apr;3(4):215-20; Sommerfelt, M.A. *et al.* "NOVEL PEPTIDE-BASED HIV-1 IMMUNOTHERAPY," Expert Opin Biol Ther. 2004 Mar;4(3):349-361; Alcaro, M.C. *et al.* "SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTION," Curr Protein Pept Sci. 2003 Aug;4(4):285-90; Smith, R.S. *et al.* "SYNTHETIC PEPTIDE ASSAYS TO DETECT HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 IN SEROPOSITIVE INDIVIDUALS," Arch Pathol Lab Med. 1990 Mar;114(3):254-8; Petrov, R.V. *et al.* "THE USE OF SYNTHETIC PEPTIDES IN THE DIAGNOSIS OF HIV INFECTIONS," Biomed Sci. 1990 Mar;1(3):239-44; Zolla-Pazner S. "IDENTIFYING EPITOPES OF HIV-1 THAT INDUCE PROTECTIVE ANTIBODIES," Nat Rev Immunol. 2004 Mar;4(3):199-210; Baillou, A. *et al.* "FINE SEROTYPING OF HUMAN IMMUNODEFICIENCY VIRUS SEROTYPE 1 (HIV-1) AND HIV-2 INFECTIONS BY USING SYNTHETIC OLIGOPEPTIDES REPRESENTING AN IMMUNODOMINANT DOMAIN OF HIV-1 AND HIV-2/SIMIAN IMMUNODEFICIENCY VIRUS," J Clin Microbiol. 1991 Jul;29(7):1387-91; McGaughey, G.B. *et al.* "PROGRESS TOWARDS THE DEVELOPMENT OF A HIV-1 GP41-DIRECTED VACCINE," Curr HIV Res. 2004 Apr;2(2):193-204).

Heterogeneous immunoassay techniques typically involve the use of a solid phase material to which the reaction product becomes bound, but may be adapted to involve the binding of non-immobilized antigens and antibodies (i.e., a solution-phase immunoassay). The reaction product is separated from excess sample, assay reagents, and other substances by removing the solid phase from the reaction mixture (e.g., by washing). One type of solid phase immunoassay that may be used in accordance with the present invention is a sandwich immunoassay. In the sandwich assay, the more analyte present in the sample, the greater the amount of label present on the solid phase. This type of assay format is generally preferred,

especially for the visualization of low analyte concentrations, because the appearance of label on the solid phase is more readily detected.

In accordance with a preferred embodiment of the present invention, a peptide of the present invention that is specifically reactive with an anti-HIV antibody is bound to a solid support (i.e., immobilized) and incubated in contact with the biological sample being tested for the presence of an anti-HIV antibody. A blocking agent may be added to reduce non-specific binding.

As will be appreciated, the peptide may be incubated with the biological sample in an unbound state and then subsequently bound to the solid support (i.e., immobilizable). The supports are then preferably extensively treated (e.g., by washing, etc.) to substantially remove non-HIV antibodies that may be present but that failed to bind to the bound peptide. In consequence of such treatment, an immune complex forms between the peptide and anti-HIV antibody.

A detectably labeled second antibody (capable of binding to the initial antibody (e.g., an anti-human IgG antibody)) is then preferably added and the support is incubated under conditions sufficient to permit the second antibody to bind to any anti-HIV antibody that may be present. The support is then preferably extensively treated (e.g., by washing, etc.) to substantially remove any unbound second antibody. If anti-HIV antibody is present in the test sample, then the two antibodies will form an immune complex with the immobilized peptide (i.e., a second antibody/anti-HIV antibody/ immobilized peptide sandwich). In such an assay, the detection of second antibody bound to the support is indicative of anti-HIV antibody in the sample being tested. Sandwich assay formats are described by Schuurs *et al.* U.S. Patent Nos. 3,791,932 and 4,016,043, and by Pankratz, *et al.*, U.S. Patent No. 5,876,935. The second antibody may be a natural immunoglobulin isolated from nonhuman species (e.g., anti-human IgG murine antibody, anti-human IgG goat antibody, anti-human IgM goat antibody, etc.), or it can be produced recombinantly or synthetically. It may be an intact immunoglobulin, or an immunoglobulin fragment (e.g., FAb, $F[Ab]_2$, etc.). As desired, other binding molecules (capable of binding to anti-HIV antibodies) may be employed in concert

with or in lieu of such second antibodies. For example, the anti-HIV antibodies can be biotinylated and the second antibody can be replaced with labeled avidin or streptavidin.

To eliminate the bound-free separation step and reduce the time and equipment needed for a chemical binding assay, a homogeneous assay format may alternatively be employed. In such assays, one component of the binding pair may still be immobilized; however, the presence of the second component of the binding pair is detected without a bound-free separation. Examples of homogeneous optical methods are the EMIT method of Syva, Inc. (Sunnyvale, CA), which operates through detection of fluorescence quenching; the laser nephelometry latex particle agglutination method of Behringwerke (Marburg, Germany), which operates by detecting changes in light scatter; the LPIA latex particle agglutination method of Mitsubishi Chemical Industries (Tokyo, Japan); the TDX fluorescence depolarization method of Abbott Laboratories (Abbott Park, IL); and the fluorescence energy transfer method of Cis Bio International (Paris, France). Any of such assays may be adapted for use in accordance with the objectives of the present invention.

The binding assay of the present invention may be configured as a competitive assay. In a competitive assay, the more anti-HIV antibody present in the test sample, the lower the amount of label present on the solid phase.

In a manner similar to the sandwich assay, the competitive assay can be conducted by providing a defined amount of a labeled anti-HIV antibody and determining whether the fluid being tested contains anti-HIV antibody that would compete with the labeled antibody for binding to the support. In such a competitive assay, the amount of captured labeled antibody is inversely proportional to the amount of analyte present in the test sample. Smith (U.S. Patent No. 4,401,764) describes an alternative competitive assay format using a mixed binding complex that can bind analyte or labeled analyte but in which the analyte and labeled analyte cannot simultaneously bind the complex. Clagett (U.S. Patent No. 4,746,631) describes an immunoassay method using a reaction chamber

in which an analyte/ligand/marker conjugate is displaced from the reaction surface in the presence of test sample analyte and in which the displaced analyte/ligand/marker conjugate is immobilized at a second reaction site. The conjugate includes biotin, bovine serum albumin, and synthetic peptides as the ligand component of the conjugate, and enzymes, chemiluminescent materials, enzyme inhibitors, and radionucleotides as the marker component of the conjugate.

Li (U.S. Patent No. 4,661,444) describes a competitive immunoassay using a conjugate of an anti-idiotype antibody and a second antibody, specific for a detectable label, in which the detectable response is inversely related to the presence of analyte in the sample. Allen (European Patent Appln. No. 177,191) describes a binding assay involving a conjugate of a ligand analog and a second reagent, such as fluorescein, in which the conjugate competes with the analyte (ligand) in binding to a labeled binding partner specific for the ligand, and in which the resultant labeled conjugate is then separated from the reaction mixture by means of solid phase carrying a binding partner for the second reagent. This binding assay format combines the use of a competitive binding technique and a reverse sandwich assay configuration; i.e., the binding of conjugate to the labeled binding member prior to separating conjugate from the mixture by the binding of the conjugate to the solid phase. The assay result, however, is determined as in a conventional competitive assay in which the amount of label bound to the solid phase is inversely proportional to the amount of analyte in the test sample. Chieregatt *et al.* (GB Patent No. 2,084,317) describe a similar assay format using an indirectly labeled binding partner specific for the analyte. Mochida *et al.* (U.S. Patent No. 4,185,084) also describe the use of a double-antigen conjugate that competes with an antigen analyte for binding to an immobilized antibody and that is then labeled. This method also results in the detection of label on a solid phase in which the amount of label is inversely proportional to the amount of analyte in the test sample. Sadeh *et al.* (U.S. Patent No. 4,243,749) describe a similar enzyme immunoassay in which a hapten conjugate competes with analyte for binding to an antibody immobilized on a solid phase. Any of such variant assays may be used in accordance with the present invention.

In all such assay formats, at least one component of the assay reagents will preferably be labeled or otherwise detectable by the evolution or quenching of light. Such component may be a second antibody, anti-HIV antibody, or the peptide that binds to the anti-HIV antibody, depending on the immunoassay format employed. Radioisotopic-binding assay formats (e.g., a radioimmunoassay, etc.) employ a radioisotope as such label; the signal is detectable by the evolution of light in the presence of a fluorescent or fluorogenic moiety (see Lucas *et al.*, U.S. Patent No. 5,698,411 and Landrum *et al.*, U.S. Patent No. 5,976,822). Enzymatic-binding assay formats (e.g., an ELISA, etc.) employ an enzyme as a label; the signal is detectable by the evolution of color or light in the presence of a chromogenic or fluorogenic moiety. Other labels, such as paramagnetic labels, materials used as colored particles, latex particles, colloidal metals such as selenium and gold, and dye particles (see U.S. Patent Nos. 4,313,734; 4,373,932, and 5,501,985) may also be employed. The use of enzymes (especially alkaline phosphatase, β-galactosidase, horse radish peroxidase, or urease) as the detectable label (i.e., an enzyme immunoassay or EIA) is preferred.

The presence of enzymatic labels may be detected through the use of chromogenic substrates (including those that evolve or adsorb fluorescent, UV, visible light, etc.) in response to catalysis by the enzyme label. More preferably, chemical labels may be employed (e.g., colloidal gold, latex bead labels, etc.). Detection of label can be accomplished using multiple detectors, multipass filters, gratings, or spectrally distinct fluors (see e.g., U.S. Patent No. 5,759,781), etc. It is particularly preferred to employ peroxidase as an enzyme label, especially in concert with the chromogenic substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB), OPD, or ABTS. In the case of labeling of the antibodies with peroxidase as enzyme, it is possible to use the periodate technique (Nakane, P.K. *et al.* (1974) "PEROXIDASE-LABELED ANTIBODY. A NEW METHOD OF CONJUGATION," J Histochem Cytochem. 22:1084-90) or a method reported in which the partners are linked with a heterobifunctional reagent (Ishikawa, E. *et al.* (1983) "ENZYME-LABELING OF ANTIBODIES AND THEIR FRAGMENTS FOR ENZYME IMMUNOASSAY AND IMMUNOHISTOCHEMICAL STAINING," J Immunoassay. 49(3):209-327).

Any of a wide variety of solid supports may be employed in the immunoassays of the present invention. Suitable materials for the solid support are synthetics such as polystyrene, polyvinyl chloride, polyamide, or other synthetic polymers, natural polymers such as cellulose, as well as derivatized natural polymers such as cellulose acetate or nitrocellulose, and glass, especially glass fibers. The support can take the form of spheres, rods, tubes, and microassay or microtiter plates. Sheet-like structures such as paper strips, small plates, and membranes are likewise suitable. The surface of the carriers can be permeable and impermeable for aqueous solutions.

Although the foregoing description pertains to assaying for the presence of anti-HIV antibodies in biological samples that are fluids (e.g., sera, blood, urine, saliva, pancreatic juice, cerebrospinal fluid, semen, etc.), it will be appreciated that any fluidic biological sample (e.g., tissue or biopsy extracts, extracts of feces, sputum, etc.) may likewise be employed in the assays of the present invention. Most preferably, the biological sample being assayed will be serum or plasma.

Table 11 illustrates the variables that may be employed in an ELISA (BSA- bovine serum albumin; FBS- fetal bovine serum; HRP-horsradish peroxidase; AP- alkaline phosphatase; TMB- 3, 3', 5, 5'-tetramethylbenzidine; OPD - o-phenylenediamine dihydrochloride).

Table 11

Epitope Presentation	Coating Amount	Blocking Agent	Serum/Plasma Dilution	2nd Antibody	2nd Antibody Dilution	Substrate
GST Protein	1,000 ng	BSA (Sigma)	1:50	HRP-Anti-Human IgG	1:1,000	Slow TMB
Peptide	500 ng	BSA (ICN)	**1:100**	HRP-Anti-Human IgG + IgM	1:2,000	Turbo TMB
Biotin-Peptide	400 ng	Gelatin		HRP-Anti-Human IgG + IgM-Fc	1:5,000	Ultra TMB
	250 ng	FBS		AP-Anti-Human IgG + IgM	**1:10,000**	**OPD (Pierce)**
	100 ng	**Milk**		AP-Anti-Human IgG + IgM-Fc	1:20,000	OPD (NEN)
	33 ng			**HRP-Anti-Human IgG-Fc**		ABTS

In a preferred embodiment of the present invention, the ELISA employs a peptide for epitope presentation, a 33 ng (for GAG-p6) or 250 ng (for Env-gp41) coating, blocking by milk, a HRP-Anti-Human IgG + IgG-Fc as the 2nd antibody (at a 10,000 fold dilution), and OPD as a substrate. Buffers that may be employed include commonly used buffers such as PBS or Tris buffers, with or without Tween-20 or other detergents commonly used for immunoassays.

Materials for use in the assay of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement; one or more containers means vials, tubes and the like; each of the containers means comprising one of the separate elements to be used in the method. For example, one of the containers means may comprise a peptide of the present invention (for example, any of **SEQ ID NOS: 1-11 or 49-55 or 141**, with or without the a peptide linker, e.g. a carboxy-terminal GGGC (**SEQ ID NO:138**) peptide linker) bound to a solid support. A second container may comprise soluble, detectably labeled second antibody, preferably in lyophilized form, or in solution. In addition, the kit may also contain one or more containers, each of which comprises a (different) predetermined amount of an anti-HIV antibody. These latter containers can be used to prepare a standard curve into which can be interpolated the results obtained from the sample containing the unknown amount of anti-HIV antibodies.

In using the kit, all the user need do is add to a container a premeasured amount of a sample suspected of containing a measurable yet unknown amount of anti-HIV antibody, a premeasured amount of support-bound peptide present in the first container, and a premeasured amount of the detectably labeled second antibody present in the second container. After an appropriate time for incubation, an immune complex is formed (if the sample contained anti-HIV antibody) and is separated from the supernatant fluid, and the immune complex or the supernatant fluid are detected, as by radioactive counting, addition of an enzyme substrate, and color development, or by inclusion of a chemical label (e.g., colloidal gold, latex beads, etc.).

The present invention particularly relates to the use of immunochromatographic assay formats to detect anti-HIV antibodies. In a preferred immunochromatographic assay format, two contacting, but spatially distinct, porous carriers are employed. The first such carrier will contain a non-immobilized, labeled peptide of the present invention and the second such carrier will contain an immobilized, but unlabeled antibody that binds to IgG (e.g., where human anti-HIV antibodies are being assayed, the unlabeled antibody may be an anti-human IgG antibody). Preferably, the device will comprise a hollow casing constructed of, for example, a plastic material, etc., in which the first carrier will communicate indirectly with the interior of the casing via a multilayer filter system that is accessible from the device (e.g., by protruding therefrom or by being incompletely covered by the device), such that a serum, plasma, or whole blood test sample can be applied directly to the filter system and will permeate therefrom into the first porous carrier. In such a device, the permeation of fluid containing anti-HIV antibodies will cause the non-immobilized labeled peptide of the first carrier to become bound to the migrating antibodies, and will then permeate into the second carrier. Because the second carrier contains immobilized antibody that binds human IgG, any labeled peptide entering the second carrier will be entrapped therein. Detection of labeled peptide in the carrier containing the immobilized unlabeled antibody thus indicates that anti-HIV antibodies were present in the sample being evaluated. The assay can be made quantitative by measuring the quantity of labeled peptide that becomes bound within the second porous carrier.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1
Identification of New Serologic Epitopes

In order to identify new serologic epitopes that conform to the above discussed criteria, gene-fragment phage display libraries are constructed by limited

DNase I digestion of HIV-1 DNA (NL4-3 clone) to generate random DNA fragments, 50-300 bp long. The fragments are purified and polished with T4 DNA polymerase and cloned at the N-termini of the coat protein of phage display vectors (**Figure 1**). This procedure results in the formation of a phage library that contains every possible encoded peptide in any of the reading frames.

The library is subjected to panning on immobilized serum antibodies from HIV-1-infected individual (early seroconversion). Phages that bind to the immobilized antibodies are retained, while non-binding, or weakly binding, phages are washed-off (**Figure 2**).

DNA sequencing of the captured phages (after amplification in and individual separation in *E. coli*) allows the mapping of the selected peptides to known HIV proteins. Initially, 11 "phagotopes" were selected and sequenced. They map to GAG-p6, RT, IN, Vif, gp120, gp41, and Nef. These sequences are produced as synthetic peptides.

Further screenings were conducted with 5 panels of sequential sera from HIV-1 seroconvertors (provided by Boston Biomedica Inc.). Four of the initial 11 epitopes are found to interact with early post-infection sera, demonstrating medium to high binding affinity. They map to GAG-p6, gp120, gp41, and Nef. Furthermore, in extended screens of random HIV seropositive plasma samples, these peptides are found to be reactive with 80-99% of all seropositive plasma tested. No false reactivity is detected with 100 sera from seronegative individuals.

Based on the above-described results, it is concluded that a combination of 1, 2, or more of the selected peptides are useful in the detection of breakthrough infections in the context of HIV vaccine trials. Sequence alignment of 3 selected peptides (from GAG-p6, gp41, and Nef) with consensus sequences for all clades in the Los Alamos database, reveals a high degree of conservation (**Figure 3**).

Example 2
Production and Use of New Serologic Epitopes

Large quantities of highly purified synthetic peptides expressing the identified sequences from HIV-1 GAG-p6, gp41, and Nef, or HIV-2 Gag-p6 and gp36, are produced under GLP conditions. The synthetic peptides are used to coat ELISA plates.

The reactivities of the synthetic peptides are tested with early seroconversion samples from different countries and different clades (under standardized conditions). Pre-clinical and clinical samples of immune sera from recent HIV vaccine candidates, likely to proceed to phase I/II/III trials, are screened in order to confirm and expand the above-described demonstration of the ability of the newly discovered HIV-1 epitopes, either alone or in combination, to react with high percentage of plasma from early seroconversion-patients. Currently licensed EIA kits may be used for side-by-side analysis.

The reactivity of immune sera from recipients of complex HIV vaccine candidates (as well as from pre-clinical studies of these vaccines) should be negative.

If plasma from Africa or Asia are not uniformly reactive with the clade B epitopes, they should score positive on consensus peptides. In addition, new Gene-fragment phage-display libraries can be constructed using HIV genomes from other clades.

The selected peptides in combination can be used to differentiate between vaccine directed antibodies and breakthrough infection generated antibodies during HIV vaccine clinical trials.

Example 3
HIV ELISA

In order to determine preferred conditions for conducting an ELISA using the peptides of the present invention, various combinations of peptides, coating amounts, blocking agents, serum/plasma dilutions, second antibodies, second

antibody dilutions and substrates are tested (a few are summarized in **Table 11**). ELISAs are characterized for the following sample types: normal (non-HIV infected) serum samples (for cut-off determination); confirmed HIV infected serum samples; seroconversion samples, serum samples from patients infected with different HIV-1 subtypes, randomly collected serum samples-blinded panel, vaccinated individual serum samples. ELISAs are also characterized for cross-reactivity with other pathogens including reteroviruses.

From such characterizations preferred assay conditions are found to employ a peptide for epitope presentation, a coating of 33ng (for GAG-p6) or 250 ng (for ENV-gp-41), blocking by 2% milk, a 1:100 serum/plasma dilution, a HRP-Anti-Human IgG -Fc as the 2nd antibody (at 1:10,000 fold dilution), and OPD as substrate. Buffers employed may be common buffers such as PBS or Tris buffer, with or without Tween-20 or other detergents commonly used for immunoassays. All incubations are done at room temperature for 1 hr each, except for blocking by milk, which is done for 2-3 hrs. The peptides employed are the GAG-p6 (**SEQ ID NO:3**) and gp41 (**SEQ ID NO:50** and **SEQ ID NO:55**)), alone and in combination. These assay conditions are employed in the ELISAs described in the examples provided below, unless otherwise indicated.

Figure 4 shows the frequency of negative samples (n=600) obtained in ELISAs employing the GAG-p6 (**SEQ ID NO:3; Panel A**) and gp41 (**SEQ ID NO:50** and **SEQ ID NO:55); Panel B**) peptides, alone and in combination (**Panel C**). For the GAG-p6 ELISA results shown in **Figure 4, panel A**, the average optical density (OD) was 0.018, the standard deviation (SD) was 0.024, and the calculated cut-off was the average optical density + 3 x SD (= 0.137). The actual cut-off used was 0.15. For the gp41 ELISA results reported in **Figure 4, Panel B**, the average optical density (OD) was 0.005, the standard deviation (SD) was 0.0025, and the calculated cut-off was the average optical density + 3 x SD (= 0.0173). The actual cut-off used was 0.035. For these ELISAs, the Specimen/Cut-Off Ratio is defined as the Absorbance of the Test Sample divided by the actual Cut-Off Value. If the Specimen/Cut-Off Ratio is greater than or equal to 1, the

sample is scored as positive for the presence of anti-HIV antibodies, and is termed "HIV positive." If the Specimen/Cut-Off Ratio is less than 1, the sample is scored as negative for the presence of anti-HIV antibodies, and is termed "HIV negative."

Using the above-described preferred assay conditions, the Specimen/Cut-Off Ratio was determined for an HIV+ serum sample at times ranging from 0 to 40 days. The results of this experiment are shown in **Table 12**. In **Table 12**, Abbott HIV1/2 is a licensed HIV serodetection kit. HIV Ag is a licensed kit to detect p24. Data relating to FDA licensed EIA Kits was generated by Boston Biomedica Inc (Gaithersburg, USA).

Table 12

Sample I.D.	Day Collected	CBER p6	CBER gp41	Abbott HIV 1/2	Abbott HIV Ag	FDA LIC. EIA KITS
PRB-929-01	**0**	0.56	0.43	0.2	0.5	0/5
PRB-929-02	**4**	0.48	0.4	0.2	0.5	0/5
PRB-929-03	**14**	0.9	0.56	0.2	0.9	0/5
PRB-929-04	**18**	0.94	0.52	0.2	**13.4**	0/5
PRB-929-05	**21**	0.8	0.5	0.9	**>22.7**	0/5
PRB-929-06	**25**	**1.2**	0.74	**>16.3**	**>22.7**	**1/5**
PRB-929-07	**28**	**8.4**	**1.02**	**>16.3**	**>22.7**	**3/5**

The seroreactivity of the peptides during acute infection was evaluated. The results of these ELISAs are shown in **Table 13**.

Table 13

Sample I.D.	Day Collected	CBER p6	CBER gp41	Abbott HIV 1/ 2	Abbott HIV Ag	FDA LIC. EIA KITS
PRB910-1	**0**	0.45	0.32	0.2	0.4	0/5
PRB910-2	**14**	0.88	0.57	0.2	**5.7**	0/5
PRB910-3	**26**	**2.8**	0.69	**10.4**	0.6	**5/5**
PRB910-4	**28**	**2.87**	0.89	**7.4**	0.5	**5/5**
PRB910-5	**32**	**4.66**	0.97	**7.6**	0.4	**5/5**
PRB910-6	**35**	**4.25**	**1.04**	**7.1**	0.4	**5/5**
PRB910-7	**40**	**3.84**	**2.01**	**7.8**	0.4	**5/5**

The analysis of five different seroconversion panels shows that HIV-1 infection can be detected using the peptides of the present invention within 2-3 weeks following HIV-1 RNA detection by the polymerase chain reaction (PCR).

Example 4
Early Detection of HIV-1 Infection by Peptides in Seroconversion Panels

The peptides and assays of the present invention are employed using the above-described ELISA conditions to detect HIV-1 infection in serum from seroconverted individuals. The data from such assays is shown in **Table 14**.

Table 14

Sample ID	ID Number	Day Collected	Specimen/Cut-Off Ratio	
			p6	gp41
AUS-105	PS04017	0	0.51	3.97
		8	0.56	5.41
		29	0.7	12.33
		56	0.87	16.24
		168	0.88	25.93
		259	2.69	29.32
AUS-107	PS01019	0	0.93	3.52
		28	1.07	4.95
		61	0.71	4.97
		168	0.78	11.95
AUS-108	PS02016	0	1.73	0.42
		12	2.64	0.45
		31	1.24	0.99
		66	3.06	4.82
		192	0.73	7.89
		269	0.77	19.35
AUS-113	PS01026	0	0.41	12.56
		27	0.56	14.91
		56	0.73	20.15
		166	0.75	21.22
AUS-114	PS01029	0	2.77	7.4
		7	2.12	7.22
		28	2.86	7.41
		56	7.63	8.18
		147	16.19	19.21
AUS-115	PM01002	0	0.84	11.49
		159	1.37	14.14
AUS-116	PM01003	0	1.11	0.61
		7	1.65	1.32
		20	1.32	2.68
		55	2.11	11.92
		168	1.28	20.92
AUS-117	PM02001	0	1.48	7.87
		7	1.24	7.43
		28	1.48	13.61
		61	0.8	19.22
		169	1.03	23.07
		252	0.82	21.89
		364	0.8	26.58
AUS-118	PM02002	0	16.07	0.81
		10	14.17	0.9

Table 14				
Sample ID	ID Number	Day Collected	Specimen/Cut-Off Ratio	
			p6	gp41
		25	12.9	4.82
		50	15.8	12.66
		170	18.21	27.58
		252	15.47	27.33
		374	7.9	30.96
AUS-120	PM02004	0	1.27	0.71
		7	1.21	0.78
		36	2.28	1.58
		70	5.34	1.98
		182	5.47	3.51
		259	5.48	7.38
AUS-121	PM02006	0	0.57	5.85
		9	0.52	4.65
		28	0.53	3.74
		56	0.69	4.39
		168	0.51	1.76
		252	0.54	1.17
AUS-123	PM03001	0	1.93	6.14
		18	1.18	4.53
		57	0.76	2.89
		87	0.71	2.63

The data demonstrate that the peptides and assays of the present invention can be employed to detect HIV-1 infection in serum from seroconverted individuals.

Example 5
Cross Clade Reactivity of Peptides for HIV-1 Diagnosis

The peptides and assays of the present invention are evaluated using the above-described ELISA conditions for cross-clade reactivity in their detection of HIV-1 infection. The data from such assays is shown in **Table 15**.

Table 15						
Sample I.D.	HIV-1 Genotype	p6	gp41	Origin of Sample	Abbott HIV 1/2	FDA LIC. EIA KITS
WWRB303-01	A	6.26	29.17	Ghana	>15.9	4/4
WWRB303-02	A	18.44	0.53	Ghana	>15.9	4/4
WWRB303-03	C	3.24	4.53	S. Africa	>15.9	4/4
WWRB303-04	C	5.12	1.09	S. Africa	>15.9	4/4
WWRB303-05	D	2.90	62.43	Uganda	>15.9	4/4
WWRB303-06	D	15.90	2.50	Uganda	>15.9	4/4

WWRB303-07	**G**	**3.02**	0.52	**Ghana**	**>15.9**	**4/4**
WWRB303-08	**G**	**1.10**	0.23	**Ivory Coast**	**>15.9**	**4/4**
WWRB303-09	**O**	0.53	0.31	**Spain**	**2.4**	**4/4**
WWRB303-10	**F**	**2.44**	0.38	**Argentina**	**>15.9**	**4/4**
WWRB303-11	**HIV-2**	0.03	0.05	**Ivory Coast**	0.3	**2/4**
WWRB303-12	**NEG**	0.03	0.07	**Argentina**	0.4	0/4
WWRB303-13	**A**	**13.60**	**50.17**	**Uganda**	**>15.9**	**4/4**
WWRB303-14	**C**	**5.37**	**1.63**	**Zimbabwe**	**>15.9**	**4/4**
WWRB303-15	**B**	0.73	**55.40**	**U.S.A**	**>15.9**	**4/4**

The data demonstrates that the peptides and assays of the present invention exhibit broad cross-clade reactivity in their detection of HIV-1 infection.

Example 6
Reactivity of Peptides with Random Serum Samples from individuals Infected with Diverse HIV-1 Clades

The peptides and assays of the present invention are evaluated using the above-described ELISA conditions for their ability to detect HIV-1 infection using random serum samples from individuals infected with diverse HIV-1 clades. The data from such assays is shown in **Table 16**.

Table 16

Sample I.D.	Subtype	p6	gp41
NYU-01	$AG^{pro}\ AG^{gag}\ A^{env}$	3.8	91.63
NYU-02	AG^{pro}	17.79	36.96
NYU-03	$AG^{pro}\ AG^{gag}\ AG^{env}$	0.75	1.53
NYU-04	$AG^{pro}\ G^{gag}\ G^{env}$	9.24	87.7
NYU-05	$AG^{pro}\ AG^{gag}\ A^{env}$	5.64	53.8
NYU-06	$AG^{pro}\ A^{gag}\ A^{env}$	19.15	91.6
NYU-07	J^{pro}	11.76	0.1
NYU-08	A^{pro}	1.84	86.8
NYU-09	$AG^{pro}\ AG^{gag}\ A^{env}$	1.6	45.43
NYU-10	AG^{pro}	1.86	0.47
NYU-11	AG^{pro}	7.52	96.57
NYU-12	AG^{pro}	14.78	6.07
NYU-13	AG^{pro}	0.26	95.87
NYU-14	AG^{pro}	0.45	6.83
NYU-15	AG^{pro}	1.3	0.33

Table 16

Sample I.D.	Subtype	p6	gp41
NYU-16	$AG^{pro}\ AG^{gag}\ AG^{env}$	0.9	88.03
NYU-17	$AG^{pro}\ AG^{gag}\ AG^{env}$	1.56	73.07
NYU-18	A^{pro}	0.4	3.73
NYU-19	$A^{pro}\ A^{gag}\ AG^{env}$	5.67	79.27
NYU-20	$A^{pro}\ A^{gag}\ A^{env}$	18.56	16.17
NYU-21	AG^{pro}	18.33	91.97
NYU-22	$A^{pro}\ A^{gag}\ A^{env}$	1.63	93.43
NYU-23	AG^{pro}	15.05	70.27
NYU-24	AG^{pro}	3.86	1.63
NYU-25	AG^{pro}	16.14	73.97
NYU-26	$AG^{pro}\ AG^{gag}\ A^{env}$	20.48	87.27
NYU-27	AG^{pro}	4.68	0.8
NYU-28	AG^{pro}	15.66	87
NYU-29	AG^{pro}	7	0.47
NYU-30	AG^{pro}	8.78	84.3
NYU-31	AG^{pro}	2.33	87.17
NYU-32	A^{pro}	0.39	1.7
NYU-33	$AG^{pro}\ F2^{gag}\ F2^{env}$	13.67	97.67
NYU-34	$AG^{pro}\ AG^{gag}\ AG^{env}$	1.48	72.5
NYU-35	AG^{pro}	4.96	15.37
NYU-36	J^{pro}	0.04	56.26
NYU-37	$G^{pro}\ G^{gag}\ G^{env}$	0.58	92.37
NYU-38	AG^{pro}	1.23	1.93
NYU-39	AG^{pro}	0.59	0.33
NYU-40	AG^{pro}	18.07	89.9
NYU-41	AG^{pro}	10.41	93.43
NYU-42	AG^{pro}	0.67	77.73
NYU-43	C^{pro}	1.15	0.57
NYU-44	$AG^{pro}\ AG^{gag}\ AG^{env}$	1.31	1.03
NYU-45	AG^{pro}	10.73	5.23
NYU-46	$A^{pro}\ AE^{gag}\ A^{env}$	9.65	72.7
NYU-47	AG^{pro}	1.71	44.7
NYU-48	AG^{pro}	8.06	90.97
NYU-49	AG^{pro}	1.04	86.23
NYU-50	AG^{pro}	2.27	92.6
NYU-51	A^{pro}	4.65	0.67
NYU-52	D^{pro}	1.84	94.5
NYU-53	$D^{pro}\ D^{gag}\ D^{env}$	2.3	35.8
NYU-54	$J^{pro}\ G^{gag}\ A^{env}$	18.24	93.03

Table 16

Sample I.D.	Subtype	p6	gp41
NYU-55	AG^{pro}	3.05	92.83
NYU-56	$G^{pro}\ G^{gag}\ A^{env}$	1.96	2
NYU-57	AG^{pro}	15.89	84.8
NYU-58	G^{pro}	1.57	8.83
NYU-59	U^{pro}	1.5	68.7
NYU-60	A^{pro}	3.18	95.5
NYU-61	A^{pro}	0.58	93
NYU-62	AG^{pro}	6.22	77.97
NYU-63	A^{pro}	1.31	96.1
NYU-64	AG^{pro}	11.61	30.3
NYU-65	A^{pro}	0.92	77.53
NYU-66	$AG^{pro}\ AG^{gag}\ A^{env}$	2.24	6.3
NYU-67	AG^{pro}	20.34	7.07
NYU-68	AG^{pro}	7.93	49.17
NYU-69	AG^{pro}	2.01	96.2
NYU-70	$AG^{pro}\ AG^{gag}\ AG^{env}$	1.29	53.17
NYU-71	AG^{pro}	20.66	9.56
NYU-72	AG^{pro}	3	12.14
NYU-73	AG^{pro}	6.27	54.16
NYU-74	A^{pro}	19.21	4.68
NYU-75	AG^{pro}	16.09	15.82
NYU-76	AG^{pro}	6.79	54.58
NYU-77	$J^{pro}\ 11cpx^{gag}\ 11cpx^{env}$	1.63	11.84
NYU-78	AG^{pro}	6.13	0.1
NYU-79	AG^{pro}	6.12	92.07
NYU-80	AG^{pro}	0.37	91.97
NYU-81	G^{pro}	3.87	25.6
NYU-82	D^{pro}	0.38	7.23
NYU-83	AG^{pro}	10.1	30.7
NYU-84	H^{pro}	0.87	97.6
NYU-85	$AG^{pro}\ AG^{gag}\ A^{env}$	1.39	2.1
NYU-86	D^{pro}	7.4	1.43
NYU-87	$F2^{pro}$	0.49	86.1
NYU-88	$A^{pro}\ AG^{gag}\ AG^{env}$	16.62	97.47
NYU-89	AG^{pro}	15.99	79.2
NYU-90	$G^{pro}\ G^{gag}\ G^{env}$	6.96	95.83
NYU-91	$AG^{pro}\ C^{gag}\ AG^{env}$	1.03	1.03
NYU-92	$J^{pro}\ 11cpx^{gag}\ A^{env}$	1.21	0.77
NYU-93	$AG^{pro}\ AG^{gag}\ AG^{env}$	1	97.9

Table 16			
Sample I.D.	**Subtype**	**p6**	**gp41**
NYU-94	$C^{pro}C^{gag}C^{env}$	5.24	0.33
NYU-95	C^{pro}	6.54	1.17
NYU-96	AG^{pro}	0.97	0.06
NYU-97	$U^{pro}AG^{gag}A^{env}$	1.03	1.22
NYU-98	AG^{pro}	20.6	2.08
NYU-99	$AG^{pro}AG^{gag}AG^{env}$	3.63	56.8
NYU-100	$F2^{pro}F2^{gag}F2^{env}$	4.89	40.8

The data shown in **Table 16** indicates that the gag p6 epitope had an individual sensitivity of 83%, compared with 67% for the gp41 epitope. The use of both epitopes had an individual sensitivity of 82%. The overall sensitivity was 100%.

Example 7
Reactivity Of Peptides Designed Based On HIV-1 Subtype Consensus Sequences With False Negative Serum Samples

The reactivity of peptides designed based on HIV-1 subtype consensus sequences with false negative serum samples (using the HIV subtype-B based peptides) is evaluated using the above-described ELISA conditions. The data is shown in **Table 17** below.

Table 17				
	Specimen/Cut-Off Ratio			
Sample ID	**p6**	**gp41**	**Consensus p6**	**Consensus gp41**
NYU-02	18.67	12.86	19.28	22.28
NYU-03	0.96	0.2	3.06	0.31
NYU-14	0.64	0.19	3.31	0.21
NYU-18	0.83	0.38	1.37	0.18
NYU-32	0.66	0.23	2.15	0.26
NYU-36	18.14	11.19	17.01	10.04
NYU-39	1.11	0.72	1.31	0.53
NYU-82	0.65	0.22	0.8	0.32
NYU-96	2.22	0.2	3.94	0.47
CBER Negative	0.49	0.1	0.36	0.12
CBER Positive	0.09	29.4	0.19	26.28
HIV Ig	7.28	42.53	6.18	36.49

The data shows that peptides designed based on HIV-1 subtype consensus sequences with false negative serum could detect HIV infection.

In summary, the data shows that HIV-1 specific peptides individually or in combination are able to detect anti-HIV-1 antibodies in serum or plasma early after acute infection. The assay specificity for the 1200 samples obtained from individuals infected with diverse HIV clades is found to be 98.2 % (For Consensus peptide GAG-p6) and 100 % (For Consensus peptide CON-Env2-gp41). The cross clade combined reactivity is found to be 94.4 % sensitivity (B-subtype) and 99.1% (for Consensus peptides). The peptides and assays of the present invention are able to detect HIV epitopes in serum samples infected with diverse HIV-1 subtypes.

Example 8
Reactivity Of Peptides With Random Serum Samples

The reactivity of the CBER p6 and CBER gp41 with a wide variety of diverse serum samples are shown in **Table 18** below.

Table 18

SAMPLE ID	CBER p6	CBER Gp41		Subtype and Infection Status
NHLBI-1	17.57	0.23		Brazil; clade B; LSI
NHLBI-2	0.98	6.22		Brazil; clade B; LSI
NHLBI-3	0.69	13.55		Brazil; clade B; LSI
NHLBI-4	1.44	27.58		Brazil; clade B; LSI
NHLBI-5	7.86	35.23		Brazil; clade B; LSI
NHLBI-6	3.18	0.43		Brazil; clade B; LSI
NHLBI-7	7.21	33.97		Brazil; clade B; LSI
NHLBI-8	4.77	1.75		Brazil; clade B; LSI
NHLBI-9	15.90	0.18		Brazil; clade B; LSI
NHLBI-10	6.34	0.35		Brazil; clade B; LSI
NHLBI-11	10.56	24.45		Brazil; clade B; LSI
NHLBI-12	2.63	7.94		Brazil; clade B; LSI
NHLBI-13	1.13	14.96		Brazil; clade B; LSI
NHLBI-14	2.03	7.58		Brazil; clade B; LSI
NHLBI-15	3.18	0.51		Brazil; clade B; LSI
NHLBI-16	15.74	6.25		Brazil; clade B; LSI
NHLBI-17	2.01	0.30		Brazil; clade B; LSI
NHLBI-18	13.14	32.63		Brazil; clade B; LSI
NHLBI-19	5.52	4.30		Brazil; clade B; LSI
NHLBI-20	1.14	0.93		Brazil; clade B; LSI
NHLBI-21	3.52	12.28		Brazil; clade B; LSI
NHLBI-22	10.25	26.03		Brazil; clade B; LSI
NHLBI-23	2.91	0.36		Brazil; clade B; LSI
NHLBI-24	6.88	0.20		Brazil; clade B; LSI
NHLBI-25	3.03	8.89		Brazil; clade B; LSI
NHLBI-26	0.95	2.89		Brazil; clade B; recent

Table 18				
SAMPLE ID	CBER p6	CBER Gp41		Subtype and Infection Status
NHLBI-27	7.90	1.94		Brazil; clade B; recent
NHLBI-28	2.45	0.35		Brazil; clade B; recent
NHLBI-29	4.73	0.57		Brazil; clade B; recent
NHLBI-30	5.21	1.64		Brazil; clade B; recent
NHLBI-31	2.60	0.55		Brazil; clade B; recent
NHLBI-32	0.73	2.1		Brazil; clade B; recent
NHLBI-33	1.63	0.25		Brazil; clade B; recent
NHLBI-34	1.67	1.78		Brazil; clade B; recent
NHLBI-35	1.20	1.16		Brazil; clade B; recent
NHLBI-36	3.72	0.12		Brazil; clade B; recent
NHLBI-37	6.59	0.43		Brazil; clade B; recent
NHLBI-38	0.63	1.40		Brazil; clade B; recent
NHLBI-39	4.32	15.82		Brazil; clade B; recent
NHLBI-40	1.57	0.31		Brazil; clade B; recent
NHLBI-41	3.19	0.43		Brazil; clade B; recent
NHLBI-42	15.65	0.77		Brazil; clade B; recent
NHLBI-43	5.44	1.91		Brazil; clade B; recent
NHLBI-44	1.13	0.39		Brazil; clade B; recent
NHLBI-45	0.89	1.39		Brazil; clade B; recent
NHLBI-46	2.86	0.93		Brazil; clade B; recent
NHLBI-47	1.13	3.13		Brazil; clade B; recent
NHLBI-48	6.11	0.37		Brazil; clade B; recent
NHLBI-49	0.65	7.13		Brazil; clade B; recent
NHLBI-50	1.97	0.50		Brazil; clade B; recent
NHLBI-51	0.42	0.23		US false positive: EIA R; WB ind
NHLBI-52	0.31	0.09		US false positive: EIA R; WB ind
NHLBI-53	0.04	0.10		US false positive: EIA R; WB ind
NHLBI-54	0.62	0.20		US false positive: EIA R; WB ind
NHLBI-55	0.06	0.13		US false positive: EIA R; WB ind
NHLBI-56	0.18	0.13		US false positive: EIA R; WB ind
NHLBI-57	0.10	0.08		US false positive: EIA R; WB ind
NHLBI-58	0.44	0.29		US false positive: EIA R; WB ind
NHLBI-59	0.23	0.18		US false positive: EIA R; WB ind
NHLBI-60	0.59	0.24		US false positive: EIA R; WB ind
NHLBI-61	0.18	0.18		US false positive: EIA R; WB ind
NHLBI-62	0.77	0.23		US false positive: EIA R; WB ind
NHLBI-63	0.30	0.18		US false positive: EIA R; WB ind
NHLBI-64	0.04	0.08		US false positive: EIA R; WB ind
NHLBI-65	0.10	0.10		US false positive: EIA R; WB ind
NHLBI-66	0.06	0.07		US false positive: EIA R; WB ind
NHLBI-67	0.13	0.21		US false positive: EIA R; WB ind
NHLBI-68	0.09	0.18		US false positive: EIA R; WB ind
NHLBI-69	0.16	0.20		US false positive: EIA R; WB ind
NHLBI-70	0.11	0.23		US false positive: EIA R; WB ind
NHLBI-71	0.10	0.14		US false positive: EIA R; WB ind
NHLBI-72	0.13	0.39		US false positive: EIA R; WB ind
NHLBI-73	0.80	0.10		US false positive: EIA R; WB ind
NHLBI-74	0.53	0.19		US false positive: EIA R; WB ind
NHLBI-75	0.16	0.41		US false positive: EIA R; WB ind
NHLBI-76	0.04	0.18		US false positive: EIA R; WB neg
NHLBI-77	0.13	0.13		US false positive: EIA R; WB neg

Table 18				
SAMPLE ID	**CBER p6**	**CBER Gp41**		**Subtype and Infection Status**
NHLBI-78	0.09	0.16		US false positive: EIA R; WB neg
NHLBI-79	0.34	0.26		US false positive: EIA R; WB neg
NHLBI-80	0.10	0.20		US false positive: EIA R; WB neg
NHLBI-81	0.37	0.18		US false positive: EIA R; WB neg
NHLBI-82	0.40	0.28		US false positive: EIA R; WB neg
NHLBI-83	0.28	0.23		US false positive: EIA R; WB neg
NHLBI-84	0.36	0.10		US false positive: EIA R; WB neg
NHLBI-85	0.12	0.55		US false positive: EIA R; WB neg
NHLBI-86	0.71	0.65		US false positive: EIA R; WB neg
NHLBI-87	0.81	0.21		US false positive: EIA R; WB neg
NHLBI-88	0.28	0.31		US false positive: EIA R; WB neg
NHLBI-89	0.12	0.20		US false positive: EIA R; WB neg
NHLBI-90	0.06	0.37		US false positive: EIA R; WB neg
NHLBI-91	0.39	0.23		US false positive: EIA R; WB neg
NHLBI-92	0.38	0.63		US false positive: EIA R; WB neg
NHLBI-93	0.36	0.53		US false positive: EIA R; WB neg
NHLBI-94	0.41	0.28		US false positive: EIA R; WB neg
NHLBI-95	0.16	0.23		US false positive: EIA R; WB neg
NHLBI-96	0.74	0.50		US false positive: EIA R; WB neg
NHLBI-97	0.09	0.40		US false positive: EIA R; WB neg
NHLBI-98	0.15	0.20		US false positive: EIA R; WB neg
NHLBI-99	0.16	0.20		US false positive: EIA R; WB neg
NHLBI-100	0.09	0.26		US false positive: EIA R; WB neg
NHLBI-101	0.17	0.11		US HIV uninfected: EIA NR; WB NT
NHLBI-102	0.19	0.49		US HIV uninfected: EIA NR; WB NT
NHLBI-103	0.45	0.56		US HIV uninfected: EIA NR; WB NT
NHLBI-104	0.11	0.13		US HIV uninfected: EIA NR; WB NT
NHLBI-105	0.11	0.17		US HIV uninfected: EIA NR; WB NT
NHLBI-106	0.22	0.17		US HIV uninfected: EIA NR; WB NT
NHLBI-107	0.35	0.28		US HIV uninfected: EIA NR; WB NT
NHLBI-108	0.32	0.43		US HIV uninfected: EIA NR; WB NT
NHLBI-109	0.61	0.1		US HIV uninfected: EIA NR; WB NT
NHLBI-110	0.26	0.44		US HIV uninfected: EIA NR; WB NT
NHLBI-111	0.19	0.12		US HIV uninfected: EIA NR; WB NT
NHLBI-112	0.41	0.13		US HIV uninfected: EIA NR; WB NT
NHLBI-113	0.14	0.33		US HIV uninfected: EIA NR; WB NT
NHLBI-114	0.20	0.36		US HIV uninfected: EIA NR; WB NT
NHLBI-115	0.13	0.31		US HIV uninfected: EIA NR; WB NT
NHLBI-116	0.50	0.44		US HIV uninfected: EIA NR; WB NT
NHLBI-117	0.26	0.43		US HIV uninfected: EIA NR; WB NT
NHLBI-118	0.19	0.30		US HIV uninfected: EIA NR; WB NT
NHLBI-119	0.21	0.34		US HIV uninfected: EIA NR; WB NT
NHLBI-120	0.17	0.36		US HIV uninfected: EIA NR; WB NT
NHLBI-121	0.20	0.42		US HIV uninfected: EIA NR; WB NT
NHLBI-122	0.20	0.26		US HIV uninfected: EIA NR; WB NT
NHLBI-123	0.29	0.41		US HIV uninfected: EIA NR; WB NT
NHLBI-124	0.15	0.30		US HIV uninfected: EIA NR; WB NT
NHLBI-125	0.48	0.84		US HIV uninfected: EIA NR; WB NT
NHLBI-126	0.27	0.62		US HIV uninfected: EIA NR; WB NT
NHLBI-127	0.57	0.11		US HIV uninfected: EIA NR; WB NT
NHLBI-128	0.17	0.66		US HIV uninfected: EIA NR; WB NT

Table 18

SAMPLE ID	CBER p6	CBER Gp41		Subtype and Infection Status
NHLBI-129	0.37	0.56		US HIV uninfected: EIA NR; WB NT
NHLBI-130	0.20	0.34		US HIV uninfected: EIA NR; WB NT
NHLBI-131	0.23	0.35		US HIV uninfected: EIA NR; WB NT
NHLBI-132	0.19	0.39		US HIV uninfected: EIA NR; WB NT
NHLBI-133	0.25	0.46		US HIV uninfected: EIA NR; WB NT
NHLBI-134	0.19	0.34		US HIV uninfected: EIA NR; WB NT
NHLBI-135	0.28	0.33		US HIV uninfected: EIA NR; WB NT
NHLBI-136	0.08	0.14		US HIV uninfected: EIA NR; WB NT
NHLBI-137	0.50	0.36		US HIV uninfected: EIA NR; WB NT
NHLBI-138	0.21	0.43		US HIV uninfected: EIA NR; WB NT
NHLBI-139	0.19	0.29		US HIV uninfected: EIA NR; WB NT
NHLBI-140	0.52	0.68		US HIV uninfected: EIA NR; WB NT
NHLBI-141	0.23	0.49		US HIV uninfected: EIA NR; WB NT
NHLBI-142	0.23	0.19		US HIV uninfected: EIA NR; WB NT
NHLBI-143	0.24	0.38		US HIV uninfected: EIA NR; WB NT
NHLBI-144	0.19	0.42		US HIV uninfected: EIA NR; WB NT
NHLBI-145	0.26	0.41		US HIV uninfected: EIA NR; WB NT
NHLBI-146	0.15	0.45		US HIV uninfected: EIA NR; WB NT
NHLBI-147	11.20	0.33		US HIV uninfected: EIA NR; WB NT
NHLBI-148	0.37	0.34		US HIV uninfected: EIA NR; WB NT
NHLBI-149	0.19	0.30		US HIV uninfected: EIA NR; WB NT
NHLBI-150	0.22	0.35		US HIV uninfected: EIA NR; WB NT
NHLBI-151	0.67	3.34		US; clade B; presumed LSI
NHLBI-152	0.47	1.90		US; clade B; presumed LSI
NHLBI-153	0.86	13.98		US; clade B; presumed LSI
NHLBI-154	0.96	21.23		US; clade B; presumed LSI
NHLBI-155	0.25	9.07		US; clade B; presumed LSI
NHLBI-156	0.25	10.32		US; clade B; presumed LSI
NHLBI-157	0.20	5.31		US; clade B; presumed LSI
NHLBI-158	6.81	0.37		US; clade B; presumed LSI
NHLBI-159	0.24	0.28		US; clade B; presumed LSI
NHLBI-160	0.83	10.63		US; clade B; presumed LSI
NHLBI-161	1.28	13.47		US; clade B; presumed LSI
NHLBI-162	0.23	3.57		US; clade B; presumed LSI
NHLBI-163	7.04	0.13		US; clade B; presumed LSI
NHLBI-164	0.10	6.87		US; clade B; presumed LSI
NHLBI-165	0.24	4.44		US; clade B; presumed LSI
NHLBI-166	0.20	9.78		US; clade B; presumed LSI
NHLBI-167	0.74	3.60		US; clade B; presumed LSI
NHLBI-168	1.66	0.40		US; clade B; presumed LSI
NHLBI-169	0.62	23.03		US; clade B; presumed LSI
NHLBI-170	8.35	0.09		US; clade B; presumed LSI
NHLBI-171	0.10	36.57		US; clade B; presumed LSI
NHLBI-172	0.41	1.3		US; clade B; presumed LSI
NHLBI-173	0.97	34.2		US; clade B; presumed LSI
NHLBI-174	2.25	2.48		US; clade B; presumed LSI
NHLBI-175	0.93	13.76		US; clade B; presumed LSI
NHLBI-176	1	0.46		US; clade B; presumed LSI
NHLBI-177	0.38	0.64		US; clade B; presumed LSI
NHLBI-178	8.93	14.48		US; clade B; presumed LSI
NHLBI-179	0.63	15.94		US; clade B; presumed LSI

Table 18				
SAMPLE ID	**CBER p6**	**CBER Gp41**		**Subtype and Infection Status**
NHLBI-180	0.40	15.36		US; clade B; presumed LSI
NHLBI-181	18.17	3.13		US; clade B; presumed LSI
NHLBI-182	0.47	1.36		US; clade B; presumed LSI
NHLBI-183	0.34	12.5		US; clade B; presumed LSI
NHLBI-184	0.37	1.42		US; clade B; presumed LSI
NHLBI-185	0.07	2.00		US; clade B; presumed LSI
NHLBI-186	0.07	11.24		US; clade B; presumed LSI
NHLBI-187	0.17	24.03		US; clade B; presumed LSI
NHLBI-188	0.27	10.29		US; clade B; presumed LSI
NHLBI-189	1.55	17.98		US; clade B; presumed LSI
NHLBI-190	0.21	9.35		US; clade B; presumed LSI
NHLBI-191	0.14	1.31		US; clade B; presumed LSI
NHLBI-192	0.16	2.73		US; clade B; presumed LSI
NHLBI-193	0.10	24.76		US; clade B; presumed LSI
NHLBI-194	11.37	0.24		US; clade B; presumed LSI
NHLBI-195	9.84	9.46		US; clade B; presumed LSI
NHLBI-196	0.60	5.82		US; clade B; presumed LSI
NHLBI-197	3.44	1.90		US; clade B; presumed LSI
NHLBI-198	18.01	9.06		US; clade B; presumed LSI
NHLBI-199	0.27	1.49		US; clade B; presumed LSI
NHLBI-200	4.10	0.72		US; clade B; presumed LSI
NHLBI-201	0.97	1.18		SA (black); clade C; recent
NHLBI-202	0.54	1.40		SA (black); clade C; recent
NHLBI-203	0.48	2.47		SA (black); clade C; recent
NHLBI-204	0.67	1.62		SA (black); clade C; recent
NHLBI-205	0.81	5.62		SA (black); clade C; recent
NHLBI-206	0.55	1.02		SA (black); clade C; recent
NHLBI-207	2.37	1.48		SA (black); clade C; recent
NHLBI-208	9.02	0.46		SA (black); clade C; recent
NHLBI-209	2.73	0.58		SA (black); clade C; recent
NHLBI-210	4.04	0.62		SA (black); clade C; recent
NHLBI-211	0.30	1.70		SA (black); clade C; recent
NHLBI-212	2.41	0.44		SA (black); clade C; recent
NHLBI-213	2.49	3.89		SA (black); clade C; recent
NHLBI-214	0.73	3.24		SA (black); clade C; recent
NHLBI-215	0.88	2.96		SA (black); clade C; recent
NHLBI-216	1.07	0.68		SA (black); clade C; recent
NHLBI-217	1.57	4.78		SA (black); clade C; recent
NHLBI-218	0.35	1.38		SA (black); clade C; recent
NHLBI-219	0.31	4.53		SA (black); clade C; recent
NHLBI-220	4.71	2.11		SA (black); clade C; recent
NHLBI-221	0.40	2.36		SA (black); clade C; recent
NHLBI-222	3.97	0.60		SA (black); clade C; recent
NHLBI-223	3.34	2.34		SA (black); clade C; recent
NHLBI-224	0.90	1.12		SA (black); clade C; recent
NHLBI-225	1.94	0.50		SA (black); clade C; recent
NHLBI-226	2.57	59.11		SA (black); clade C; LSI
NHLBI-227	3.18	0.40		SA (black); clade C; LSI
NHLBI-228	0.75	55.56		SA (black); clade C; LSI
NHLBI-229	1.03	31.31		SA (black); clade C; LSI
NHLBI-230	2.13	0.58		SA (black); clade C; LSI

Table 18				
SAMPLE ID	**CBER p6**	**CBER Gp41**		**Subtype and Infection Status**
NHLBI-231	0.40	16.64		SA (black); clade C; LSI
NHLBI-232	0.37	51.67		SA (black); clade C; LSI
NHLBI-233	2.87	0.90		SA (black); clade C; LSI
NHLBI-234	0.17	54.16		SA (black); clade C; LSI
NHLBI-235	0.43	1.60		SA (black); clade C; LSI
NHLBI-236	1.04	0.86		SA (black); clade C; LSI
NHLBI-237	2.59	46.40		SA (black); clade C; LSI
NHLBI-238	1.09	3.51		SA (black); clade C; LSI
NHLBI-239	2.26	10.00		SA (black); clade C; LSI
NHLBI-240	0.75	21.18		SA (black); clade C; LSI
NHLBI-241	1.44	1.02		SA (black); clade C; LSI
NHLBI-242	0.79	1.06		SA (black); clade C; LSI
NHLBI-243	1.40	3.22		SA (black); clade C; LSI
NHLBI-244	0.53	9.87		SA (black); clade C; LSI
NHLBI-245	0.58	3.11		SA (black); clade C; LSI
NHLBI-246	15.40	1.84		SA (black); clade C; LSI
NHLBI-247	1.33	9.62		SA (black); clade C; LSI
NHLBI-248	0.31	9.36		SA (black); clade C; LSI
NHLBI-249	1.86	47.27		SA (black); clade C; LSI
NHLBI-250	0.57	20.16		SA (black); clade C; LSI
NHLBI-251	2.14	5.42		US (ARC); clade B; recent
NHLBI-252	0.66	1.50		US (ARC); clade B; recent
NHLBI-253	10.06	1.00		US (ARC); clade B; recent
NHLBI-254	1.13	0.58		US (ARC); clade B; recent
NHLBI-255	0.60	1.02		US (ARC); clade B; recent
NHLBI-256	1.49	5.47		US (ARC); clade B; recent
NHLBI-257	3.25	12.07		US (ARC); clade B; recent
NHLBI-258	3.86	1.62		US (ARC); clade B; recent
NHLBI-259	1.78	25.64		US (ARC); clade B; recent
NHLBI-260	1.09	1.58		US (ARC); clade B; recent
NHLBI-261	0.18	5.36		US (ARC); clade B; recent
NHLBI-262	1.99	15.73		US (ARC); clade B; recent
NHLBI-263	6.14	2.02		US (ARC); clade B; recent
NHLBI-264	9.39	1.06		US (ARC); clade B; recent
NHLBI-265	0.55	0.64		US (ARC); clade B; recent
NHLBI-266	1.68	30.49		US (ARC); clade B; recent
NHLBI-267	1.09	0.56		US (ARC); clade B; recent
NHLBI-268	1.50	0.48		US (ARC); clade B; recent
NHLBI-269	3.43	7.08		US (ARC); clade B; recent
NHLBI-270	0.43	4.11		US (ARC); clade B; recent
NHLBI-271	0.50	3.56		US (ARC); clade B; recent
NHLBI-272	0.27	1.12		US (ARC); clade B; recent
NHLBI-273	1.03	0.74		US (ARC); clade B; recent
NHLBI-274	5.41	13.78		US (ARC); clade B; recent
NHLBI-275	1.06	2.20		US (ARC); clade B; recent
NHLBI-276	3.55	2.64		US (ARC); clade B; recent
NHLBI-277	18.51	55.93		US (ARC); clade B; recent
NHLBI-278	0.56	0.30		US (ARC); clade B; recent
NHLBI-279	1.82	17.11		US (ARC); clade B; recent
NHLBI-280	0.64	4.96		US (ARC); clade B; recent
NHLBI-281	1.20	1.62		US (ARC); clade B; recent

Table 18				
SAMPLE ID	**CBER p6**	**CBER Gp41**		**Subtype and Infection Status**
NHLBI-282	9.25	0.42		US (ARC); clade B; recent
NHLBI-283	0.39	1.20		US (ARC); clade B; recent
NHLBI-284	1.85	0.47		US (ARC); clade B; recent
NHLBI-285	0.29	5.87		US (ARC); clade B; recent
NHLBI-286	0.52	4.00		US (ARC); clade B; recent
NHLBI-287	0.83	1.16		US (ARC); clade B; recent
NHLBI-288	2.60	2.70		US (ARC); clade B; recent
NHLBI-289	2.54	0.44		US (ARC); clade B; recent
NHLBI-290	1.90	1.36		US (ARC); clade B; recent
NHLBI-291	4.04	22.56		US (ARC); clade B; recent
NHLBI-292	0.41	1.50		US (ARC); clade B; recent
NHLBI-293	3.22	3.18		US (ARC); clade B; recent
NHLBI-294	0.80	1.64		US (ARC); clade B; recent
NHLBI-295	1.28	0.37		US (ARC); clade B; recent
NHLBI-296	1.04	1.22		US (ARC); clade B; recent
NHLBI-297	7.88	0.76		US (ARC); clade B; recent
NHLBI-298	1.55	8.62		US (ARC); clade B; recent
NHLBI-299	2.15	0.64		US (ARC); clade B; recent
NHLBI-300	1.36	0.47		US (ARC); clade B; recent

Table 19 summarizes the ability of the HIV-SELECTEST to detect HIV-infected serum with diverse clades.

Table 19 **Summary of HIV-SELECTEST with HIV-Infected Serum Samples with Diverse Clades**			
Subtype	**Number of Samples**	**HIV-SELECTEST Reactive**	**% Combined Sensitivity**
A	25	25	**100**
B	263	259	98.48
C	50	50	**100**
D	20	19	95
E	104	103	99.04
G	11	11	**100**
J	4	4	**100**
AG	150	150	**100**
AJ	7	7	**100**
F2, U, H, J/G Recombinants	9	9	**100**
CRF	21	21	**100**
Unknown Genotypes	589	585	99.32
Total No. Samples	**1253**	**1243**	**99.2**

Example 8
Reactivity Of Peptides With RV124 Vaccine Trial Samples: A Comparison With the BioRad Kit

The reactivity of CBER p6 and CBER p41 with RV124 vaccine trial samples were compared with the reactivity of the BioRad Kit with RV124 vaccine trial samples at 0 and 182 days post-vaccination. The vaccination given in the RV124 vaccine trials was as follows: ALVAC-HIV (vCP205; gag-LAI + pro-LAI + gp120MN/gp41TM-LAI) with HIV-1 gp160 protein boost (gp120MN, gp41 LAI-2) (with Gag-p6). The results for individual samples are shown in **Table 20** below. The results show substantially more false positive reactions using the BioRad kit versus the use of CBER p6 or CBER p41.

Table 20
Reactivity Of RV-124 Clinical Trial Samples In The 'HIV-Selectest'

	Day 0		Day 182			Day 0	Day 182
SERUM SAMPLE	**New HIV ELISA'**		**New HIV ELISA'**		**HIV INFECTION**	**BioRad**	**BioRad**
	p6	**gp41**	**p6**	**gp41**	**STATUS**	**HIV-1/2 plus O**	**HIV-1/2 plus O**
RV124-1	0.09	0.15	0.36	0.21	NEGATIVE	0.14	**11.42**
RV124-2	0.08	0.10	0.18	0.19	NEGATIVE	0.12	**11.14**
RV124-3	0.32	0.15	0.61	0.28	NEGATIVE	0.15	**11.45**
RV124-4	0.16	0.15	0.35	0.18	NEGATIVE	0.14	**11.40**
RV124-5	0.11	0.16	0.17	0.13	NEGATIVE	0.16	**11.08**
RV124-6	0.10	0.14	0.14	0.15	NEGATIVE	0.16	**11.78**
RV124-7	0.11	0.18	0.12	0.20	NEGATIVE	0.24	**11.96**
RV124-8	0.39	0.22	0.33	0.14	NEGATIVE	0.23	**12.01**
RV124-9	0.13	0.13	0.16	0.13	NEGATIVE	0.27	**11.72**
RV124-10	0.10	0.12	0.13	0.13	NEGATIVE	0.13	**11.69**
RV124-11	0.15	0.18	0.25	0.28	NEGATIVE	0.46	**10.93**
RV124-12	0.09	0.08	0.22	0.19	NEGATIVE	0.47	**11.38**
RV124-13	0.37	0.09	0.59	0.22	NEGATIVE	0.14	**11.54**
RV124-14	0.16	0.19	0.26	0.18	NEGATIVE	0.11	**11.19**
RV124-15	0.19	0.10	0.25	0.16	NEGATIVE	0.10	**11.51**
RV124-16	0.16	0.16	0.20	0.19	NEGATIVE	0.13	**1.97**
RV124-17	0.09	0.05	0.17	0.10	NEGATIVE	0.17	**11.38**
RV124-18	0.30	0.08	0.43	0.16	NEGATIVE	0.13	**11.65**

Table 20
Reactivity Of RV-124 Clinical Trial Samples In The 'HIV-Selectest'

	Day 0		Day 182			Day 0	Day 182
SERUM SAMPLE	New HIV ELISA'		New HIV ELISA'		HIV INFECTION	BioRad	BioRad
	p6	gp41	p6	gp41	STATUS	HIV-1/2 plus O	HIV-1/2 plus O
RV124-19	0.26	0.13	0.04	0.09	NEGATIVE	0.20	**12.17**
RV124-20	0.21	0.10	0.19	0.10	NEGATIVE	0.20	**11.96**
RV124-21	0.16	0.07	0.18	0.10	NEGATIVE	0.22	**11.31**
RV124-22	0.30	0.23	0.67	0.43	NEGATIVE	0.27	**11.13**
RV124-23	0.94	0.15	0.41	0.22	NEGATIVE	0.37	0.24
RV124-24	0.15	0.11	0.51	0.42	NEGATIVE	0.48	**11.04**
RV124-25	0.44	0.09	0.48	0.21	NEGATIVE	0.15	**11.53**
RV124-26	0.09	0.08	0.41	0.22	NEGATIVE	0.14	**11.26**
RV124-27	0.24	0.42	0.34	0.47	NEGATIVE	0.14	**11.71**
RV124-28	0.14	0.11	0.32	0.20	NEGATIVE	0.15	**11.96**
RV124-29	0.16	0.79	**13.53**	0.50	NEGATIVE	0.19	**11.68**
RV124-30	0.80	0.15	0.22	0.17	NEGATIVE	0.15	**11.56**
RV124-31	0.17	0.14	0.24	0.17	NEGATIVE	0.27	**12.00**
RV124-32	0.20	0.15	0.28	0.14	NEGATIVE	0.21	**11.89**
RV124-33	0.43	0.29	0.37	0.18	NEGATIVE	0.30	**11.59**
RV124-34	0.05	0.06	0.43	0.17	NEGATIVE	0.31	**11.40**
RV124-35	0.04	0.03	**1.56**	0.27	NEGATIVE	0.59	0.11
RV124-36	0.13	0.10	0.77	0.37	NEGATIVE	0.52	**11.55**
RV124-37	0.23	0.28	0.26	0.29	NEGATIVE	0.13	0.13
RV124-38	0.31	0.38	0.45	0.33	NEGATIVE	0.12	**11.12**
RV124-39	0.31	0.20	0.47	0.19	NEGATIVE	0.11	**11.64**
RV124-40	0.27	0.16	0.54	0.22	NEGATIVE	0.15	**11.42**
RV124-41	0.30	0.18	0.43	0.19	NEGATIVE	0.38	**10.64**
RV124-42	0.37	0.23	0.96	0.20	NEGATIVE	0.21	**10.65**
RV124-43	0.53	0.21	0.50	0.24	NEGATIVE	0.26	0.14
RV124-44	0.18	0.37	0.13	0.23	NEGATIVE	0.24	**10.95**
RV124-45	0.10	0.36	0.08	0.22	NEGATIVE	0.31	**10.99**
RV124-46	0.20	0.18	0.15	0.10	NEGATIVE	0.41	**10.13**
RV124-47	0.41	0.20	0.99	0.17	NEGATIVE	0.35	**10.81**
RV124-48	0.32	0.19	0.75	0.17	NEGATIVE	0.33	**10.40**
RV124-49	0.67	0.30	0.47	0.16	NEGATIVE	0.35	0.14
RV124-50	0.63	0.18	0.43	0.11	NEGATIVE	0.35	**10.55**

Table 20
Reactivity Of RV-124 Clinical Trial Samples In The 'HIV-Selectest'

	Day 0		Day 182			Day 0	Day 182
SERUM SAMPLE	New HIV ELISA'		New HIV ELISA'		HIV INFECTION	BioRad	BioRad
	p6	gp41	p6	gp41	STATUS	HIV-1/2 plus O	HIV-1/2 plus O
RV124-51	0.22	0.09	0.99	0.09	NEGATIVE	0.46	**10.43**
RV124-52	0.87	0.33	0.23	0.27	NEGATIVE	0.32	**10.68**
RV124-53	0.63	0.18	0.52	0.25	NEGATIVE	0.20	**11.07**
RV124-54	0.26	0.13	0.53	0.13	NEGATIVE	0.15	**1.17**
RV124-55	0.27	0.14	0.27	0.12	NEGATIVE	0.16	0.19
RV124-56	0.85	0.11	0.98	0.20	NEGATIVE	0.15	**10.89**
RV124-57	0.36	0.29	0.74	0.25	NEGATIVE	0.17	0.48
RV124-58	0.23	0.20	0.73	0.20	NEGATIVE	0.22	**10.90**
RV124-59	0.40	0.37	0.45	0.20	NEGATIVE	0.22	0.53
RV124-60	0.71	0.25	0.12	0.36	NEGATIVE	0.20	**10.66**
RV124-61	0.67	0.18	0.66	0.11	NEGATIVE	0.19	0.36
RV124-62	0.52	0.47	0.43	0.37	NEGATIVE	0.16	0.15
RV124-63	0.01	0.07	0.64	0.35	NEGATIVE	0.21	**10.30**
RV124-64	0.16	0.08	0.10	0.12	NEGATIVE	0.26	0.17
RV124-65	0.13	0.30	0.21	0.18	NEGATIVE	0.23	0.19
RV124-66	0.26	0.23	0.24	0.24	NEGATIVE	0.19	0.18
RV124-67	0.64	0.20	0.41	0.10	NEGATIVE	0.19	**10.52**
RV124-68	0.48	0.29	0.44	0.21	NEGATIVE	0.26	0.27
RV124-69	0.30	0.17	0.17	0.11	NEGATIVE	0.18	0.39
RV124-70	0.31	0.25	0.30	0.11	NEGATIVE	0.16	**10.86**
RV124-71	0.37	0.18	0.45	0.18	NEGATIVE	0.21	**10.45**
RV124-72	0.32	0.19	0.84	0.15	NEGATIVE	0.23	**9.66**
RV124-73	0.41	0.23	0.63	0.11	NEGATIVE	0.22	**9.97**
RV124-74	0.32	0.23	0.28	0.31	NEGATIVE	0.21	**10.45**
RV124-75	0.27	0.08	0.44	0.12	NEGATIVE	0.22	**9.37**
RV124-76	0.47	0.16	0.47	0.20	NEGATIVE	0.31	**10.95**
RV124-77	0.49	0.10	0.37	0.29	NEGATIVE	0.17	**10.67**
RV124-78	0.04	0.24	0.07	0.20	NEGATIVE	0.15	**5.88**
RV124-79	0.32	0.20	0.25	0.18	NEGATIVE	0.15	0.14

Example 8
Reactivity Of Peptides With VRC-004 & VRC-006 Vaccine Trial Samples: A Comparison With the BioRad Kit

The reactivity of CBER p6 and CBER p41 with VRC-004 and VRC-006 vaccine trial samples are compared with the reactivity of the BioRad Kit with VRC-004 and VRC-006 vaccine trial samples. The results are shown in **Table 21** below. The results show substantially more false positive reactions using the BioRad kit versus the use of CBER p6 or CBER p41.

Table 21

SAMPLE ID	p6	gp41	HIV INFECTION STATUS	FDA LICENSED EIA
VRC-004 trial- 4 PLASMIDS -pGag-Pol-Nef + pEnv A, pEnv B + pEnv C				
004-001-05	0.07	0.1	Negative (-)	Negative (-)
004-002-05	0.03	0.1	Negative (-)	Negative (-)
004-003-08	0.01	**15.73**	***Positive (+)***	Negative (-)
004-004-05	0.05	0.07	Negative (-)	Negative (-)
004-005-05	0.02	0.1	Negative (-)	Negative (-)
004-006-05	0.02	0.07	Negative (-)	Negative (-)
004-007-05	0.03	0.07	Negative (-)	Negative (-)
004-008-05	0.07	0.1	Negative (-)	Negative (-)
004-009-05	0.03	0.07	Negative (-)	***Positive (+)***
004-010-05	0.04	0.1	Negative (-)	***Positive (+)***
004-011-05	0.05	0.13	Negative (-)	Negative (-)
004-012-05	0.03	0.03	Negative (-)	***Positive (+)***
004-013-05	0.03	0.07	Negative (-)	Negative (-)
004-014-05	0.03	0.13	Negative (-)	Negative (-)
004-015-05	0.04	0.1	Negative (-)	Negative (-)
004-016-05	0.04	0.1	Negative (-)	Negative (-)
004-017-05	0.05	0.1	Negative (-)	Negative (-)
004-018-05	0.03	0.1	Negative (-)	Negative (-)
004-019-05	0.04	0.2	Negative (-)	Negative (-)
004-020-05	**2.51**	**1.13**	***Positive (+)***	***Positive (+)***
004-021-05	0.01	0.03	Negative (-)	Negative (-)
004-022-05	0.02	0.13	Negative (-)	Negative (-)
004-023-05	0.01	0.03	Negative (-)	Negative (-)
004-024-05	0.03	0.2	Negative (-)	***Positive (+)***
004-025-05	0.03	0	Negative (-)	***Positive (+)***
004-026-05	0.1	0.23	Negative (-)	Negative (-)

Table 21				
SAMPLE ID	**p6**	**gp41**	**HIV INFECTION STATUS**	**FDA LICENSED EIA**
004-027-05	0.02	0.13	Negative (-)	Negative (-)
004-028-05	0.02	0.1	Negative (-)	Negative (-)
004-029-05	0.05	0.1	Negative (-)	Negative (-)
004-030-05	0.03	0.07	Negative (-)	Negative (-)
004-031-05	0.21	0.13	Negative (-)	Negative (-)
004-032-05	0.01	0.07	Negative (-)	Negative (-)
004-033-05	0.03	0.1	Negative (-)	Negative (-)
004-034-05	0.01	0.07	Negative (-)	Negative (-)
004-035-05	0.03	0.13	Negative (-)	***Positive (+)***
004-036-05	0.04	0.1	Negative (-)	***Positive (+)***
004-037-05	0.05	0.07	Negative (-)	***Positive (+)***
004-038-05	0.05	0.03	Negative (-)	***Positive (+)***
004-039-05	0.19	0.03	Negative (-)	Negative (-)
004-040-05	0.05	0.13	Negative (-)	***Positive (+)***
004-041-05	0.02	0.07	Negative (-)	Negative (-)
004-042-05	0.61	0.07	Negative (-)	Negative (-)
004-043-05	0.11	0.03	Negative (-)	Negative (-)
004-044-05	0.06	0.03	Negative (-)	Negative (-)
004-045-05	0.04	0.03	Negative (-)	Negative (-)
004-046-05	0.12	0.13	Negative (-)	Negative (-)
004-047-05	0.05	0.03	Negative (-)	Negative (-)
004-048-05	0.06	0.1	Negative (-)	Negative (-)
004-049-05	0.13	0.13	Negative (-)	Negative (-)
004-050-05	0.03	0.03	Negative (-)	Negative (-)
VRC-006 trial- 4 Ad5 -Ad5-Gag-Pol + Ad5-Env A, Ad5-Env B + Ad5-Env C				
006-001-03	0.19	0.2	Negative (-)	Negative (-)
006-002-03	0.01	-0.07	Negative (-)	Negative (-)
006-003-03	0.44	0.1	Negative (-)	Negative (-)
006-004-03	0.03	0.13	Negative (-)	***Positive (+)***
006-005-03	0.01	0.07	Negative (-)	Negative (-)
006-006-03	0.04	0.07	Negative (-)	Negative (-)
006-007-03	0.05	0.17	Negative (-)	Negative (-)
006-008-03	0.01	0.1	Negative (-)	Negative (-)
006-009-03	0.02	0.1	Negative (-)	Negative (-)
006-010-03	0.04	0.07	Negative (-)	***Positive (+)***
006-011-03	0.04	0.07	Negative (-)	***Positive (+)***
006-012-03	0.02	0.03	Negative (-)	Negative (-)
006-013-03	0.03	0.07	Negative (-)	Negative (-)

Table 21				
SAMPLE ID	**p6**	**gp41**	**HIV INFECTION STATUS**	**FDA LICENSED EIA**
006-014-03	0.01	0.03	Negative (-)	***Positive (+)***
006-015-03	0.28	0.1	Negative (-)	***Positive (+)***
006-016-03	0.07	0.07	Negative (-)	***Positive (+)***
006-017-03	0.03	0.07	Negative (-)	Negative (-)
006-018-03	0.01	0.03	Negative (-)	***Positive (+)***
006-019-03	0.03	0.03	Negative (-)	***Positive (+)***
006-020-03	0.05	0.1	Negative (-)	***Positive (+)***
006-021-03	0.01	0.03	Negative (-)	Negative (-)
006-022-03	0.03	0.07	Negative (-)	Negative (-)
006-023-03	0.04	0.1	Negative (-)	Negative (-)
006-024-03	0.1	0.07	Negative (-)	Negative (-)
006-025-03	0.05	0.1	Negative (-)	Negative (-)
006-026-03	0.03	0.13	Negative (-)	***Positive (+)***
006-027-03	0.01	0.07	Negative (-)	***Positive (+)***
006-028-03	0.07	0.2	Negative (-)	***Positive (+)***
006-029-03	0.03	0.1	Negative (-)	***Positive (+)***
006-030-03	0.05	0.13	Negative (-)	Negative (-)
006-031-03	0.03	0.13	Negative (-)	***Positive (+)***
006-032-03	0.03	0.1	Negative (-)	***Positive (+)***
006-033-03	0.02	0.07	Negative (-)	***Positive (+)***
006-034-03	0.03	0.1	Negative (-)	***Positive (+)***
006-035-03	0.03	0.13	Negative (-)	Negative (-)
006-036-03	0.02	0.13	Negative (-)	***Positive (+)***
SUMMARY	**1/86**	**2/86**	**2/86**	**29/86**

Example 9
The "HIV-SELECTEST"
A New HIV-1 Detection Assay

The present invention provides a new HIV-1 detection assay, in which vaccine-generated antibodies will not cross-react, while seroconversion can be detected early post-infection. The selection criteria for HIV sequences to be used in such an assay included epitopes that are: 1) not included in HIV vaccines, 2) recognized by antibodies early after HIV infection, and 3) highly conserved among HIV clades and subtypes.

To identify such sequences, a Gene-Fragment Phage Display Library was constructed from the entire HIV-1 genome and used for screening of sera from HIV-infected individuals around the time of seroconversion. As discussed above, this strategy led to the discovery of three novel epitopes, one in Gag p6 and two in the gp41 cytoplasmic tail. The development of a new HIV enzyme-linked immunosorbent assay, termed HIV-SELECTEST, which distinguishes between HIV infected individuals and uninfected vaccine recipients is described below. HIV-SELECTEST is a low cost, high throughput assay that could be implemented in clinical sites and blood collection centers worldwide, and serve as an important diagnostic tool in HIV vaccine trials.

METHODS

Construction Of A Complete HIV Genome Gene-Fragment Phage Display Library

Plasmid pNL4-3, containing the complete HIV-1 NL4-3 proviral DNA was obtained from the NIH AIDS Research and Reference Reagent Program (McKesson BioServices Corp., Rockville, MD). Full length HIV-1 genome was PCR amplified from pNL4-3 DNA with the Expand long template polymerase preparation (Roche Diagnostics, Indianapolis, IN) and primers spanning the Lys t-RNA primer binding site (MSF12, (**SEQ ID NO:139**) 5'-**AAAAATCTCTAGCAGTGGCGCCCGAACAG**-3') and the poly-A signal region of 3'-LTR (MSR5, (**SEQ ID NO:140**) 5'-**AAGCACTCAAGGCAAGCTTTATTGAGGCT**-3'), which amplifies the entire HIV-1 genome except for 75 bp in the unique-5' (U5) region of the LTR. The purified amplified DNA product was digested with DNase I using DNase shotgun cleavage kit (Novagen, Madison, WI), and fragments between 50 and 300 bp were isolated by preparative gel electrophoresis, treated with T4 DNA polymerase to generate blunt ends, and dephosphorylated using calf intestinal alkaline phosphatase (CIP) (Roche Diagnostics, Indianapolis, IN). DNA was again purified using nucleotide removal kit (Qiagen Inc, Valencia, CA) and was ligated in the presence of *Srf* I enzyme into the *Sma* I site of the M13 derived phage vector for expression as gIIIp fusion protein, followed by

electroporation into *E. coli* TG1 cells. Tet-resistant transformants were harvested and expanded in liquid culture (2X-YT) at 37 °C. The cell-free phage supernatant was isolated by centrifugation and phage titer was determined as Tet^r transduction units. Ninety six individual clones were isolated and DNA inserts were amplified by standard PCR and sequenced to determine the insert size distribution and library diversity.

Selection Of Phages Reactive With HIV Antibodies From Early Infected Individuals

Seven plasma samples constituting the HIV-1 seroconversion panel PRB-910 from SeraCare BioServices (Gaithersburg, MD) were used for panning of the HIV-1 gene-fragment phage display library (GFPDL). For removal of plasma components, which could non-specifically interact with phage proteins, 5-fold diluted plasma was pre-adsorbed three times on sterile polystyrene Petri dishes (35 mm diameter) coated with 10^{13} UV-killed VCSM13. For biopanning, the microtiter strips (NUNC Inc, Naperville, IL) were coated with a mixture of 500 ng each of goat anti-human IgG-Fc and goat anti-human IgM-Fc specific antibodies in PBS, pH 7.4. After three washings with PBST (20 mM PBS containing 0.1% Tween 20), DMEM containing 5% FBS (blocking solution) was added to wells to block the unoccupied reactive sites. VCSM13 pre-adsorbed HIV-1 human plasma was added to the wells and incubated for 1 h at room temperature (RT). Wells were washed thrice with PBST and 10^{10} phages per well of the HIV-1 GFPDL, diluted in blocking solution, were added for 2 h at RT. The unbound phages were removed in twelve washes with PBST followed by three washes with PBS. Bound phages were eluted by addition of 0.1 N HCl containing BSA (1 mg/ml), for 10 min at RT, and neutralized by adding 8 µl of 2 M Tris solution per 100 µl eluate. Four rounds of affinity selection were carried out with each individual serum sample comprising the HIV seroconversion panel PRB-910.

Analysis of Affinity Selected Phage Clones

Twenty two phage clones enriched after four rounds of biopanning on each PRB-910 plasma sample were further screened for specific recognition by HIV

seropositive sera and absence of reactivity with seronegative sera in affinity-capture phage ELISA. The wells of ELISA plates (Immulon 2HB, Thermo Labsystems, Franklin, MA) were coated with 100 ng/well of anti-phage antibody (GE Healthcare, Piscataway, NJ), and blocked with DMEM/5% FBS. Subsequently, 10^{10} phages of the selected clones were added per well and incubated for 1 h at RT. Serially diluted sera (in DMEM/5% FBS) were added to the 96-well plates in duplicate and incubated at RT for 1 h. The bound antibodies were probed with HRP-conjugated goat anti-human IgG-IgM antibodies and the reactions were developed with OPD substrate solution (Pierce Biotechnology, Rockford, IL). The clones demonstrating the best differential reactivity with HIV-1 seropositive sera were expanded and the inserts were sequenced and mapped to individual HIV-1 genes. Several inserts were selected for synthetic peptide synthesis and development of the HIV-SELECTEST.

Peptides Used In New HIV-SELECTEST

Peptide sequences from Gag-p6 (**SEQ ID NO:3**; 452-**SRPEPTAPPAESFRFGEEITPTPSQKQEPKDKELYPPLASLRSLFGNDPSSQ**-502) and gp41 cytoplasmic region (SK1; **SEQ ID NO:50**; 784-**LIAARIVELLGHSSLKGLRRGWEALKYLWNLLQYWGQELKNSAISL**-829 and SK2; **SEQ ID NO:55**; 836-**AVAEGTDRVIEVVQRVCRAILNIPRRIRQGFERALL**-871) were chemically synthesized (amino acid residues are numbered based on the CON-OF-CONS alignment sequence in the Los Alamos database). All peptides were synthesized at the Facility for Biotechnology Resources, CBER, FDA, on Applied Biosystems peptide synthesizer models 431 and 433 (Foster City, California) by standard 9-fluorenyl methoxycarbonyl chemistry (Fmoc). Peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) and characterized by mass spectrometry (MALDI-TOF MS).

HIV-SELECTEST

Based on preliminary screening of HIV seronegative and seropositive sera, the optimal conditions for the p6 and gp41 ELISA were determined. The p6 peptide was coated at 30 ng/100 µl/well while the gp41 peptides (**SEQ ID NO:50**

and **SEQ ID NO:55**) were coated at 150 ng/100 µl/well each (total 300 ng/well) on Immulon-2HB plates. After three washes with PBST (20 mM PBS, 0.1% Tween-20), the unoccupied reactive sites were blocked by PBST containing 2% whole milk (2% WMPBST). All specimens (serum or plasma) were diluted 1:100 in 2% WMPBST, added to peptide-coated wells, and incubated for 1 h at RT. The plates were then washed six times with PBST and 100 µl/well of HRP-conjugated goat anti-Human IgG Fc-specific antibody (Jackson ImmunoResearch, West Grove, PA), diluted 1:10,000 in 2% WMPBST was added. The reactions were quantified using O-Phenylenediamine (OPD) substrate.

Based on the results with 1000 seronegative samples, cut-off values were determined for p6 and gp41 peptides individually. The cut-off values used are the average absorbance of Negative sera + 5 Standard Deviations (for each peptide). Specimens with an Absorbance / Cut-off ratios of ≥ 1 are considered HIV-1 seropositive and those with ratios < 1 are considered HIV-1 seronegative.

HIV Seroconversion Panels And Vaccine Trial Samples

HIV-1 seroconversion panels PRB-910, PRB-924, PRB-927, PRB-928, PRB-929, PRB-931 and mixed titer panel PRB-204 were purchased from SeraCare BioServices, (Gaithersburg, MD). A seroconversion panel consists of plasma samples collected serially early after HIV-1 infection, and the virological and immunological profiles as assessed by commercial diagnostic kits for these plasma samples were provided by SeraCare BioServices. Additionally, twenty eight seroconversion panels were provided by the University of New South Wales (PHAEDRA Inventory, Sydney, Australia). HIV negative serum samples were obtained from National Institutes of Health Blood Bank and the Vaccine Research Center (VRC, NIAID, NIH, Bethesda, MD).

Serum/Plasma samples from the following HIV vaccine trials were tested: HVTN 203 (246 vaccinees and 78 placebos; conducted by the HIV Vaccine Trial Network), RV124 (conducted by the Walter Reed Army Institute of Research), VRC 004 (40 vaccinees and 10 placebos), VRC 006 (30 vaccinees and 6 placebos),

VRC 009 (9 vaccinees and no placebos) and VRC 010 were conducted by the Vaccine Research Center (NIAID, NIH), VAX 003 and VAX 004 were conducted by VaxGen Inc. The HIV infection status of a given sample was provided by the collaborating groups and also determined by in house testing using the BioRad HIV-1/2 plus O kit (Bio Rad laboratories, Woodinwille, WA). Samples obtained from VRC 009 and VRC 010 trials were also tested with the Capillus HIV-1/HIV-2 and Uni-Gold HIV rapid tests (Trinity Biotech, NY).

Identification Of HIV Sequences Recognized By Early Seroconversion Sera Using Gene-Fragment Phage Display Library

In order to identify all the HIV sequences recognized by antibodies generated soon after HIV infection, a gene-fragment phage display library (GFPDL) was constructed spanning the entire HIV-1 open reading frame of NL4-3. The HIV-1 GFPDL, contained more than 10^7 independent transformants. PCR-based analysis and sequencing of the inserts confirmed that the library consisted of 100% recombinants, with an insert size of 50-300 bp, and random distribution across the HIV genome.

Seven plasma samples constituting a seroconversion panel PRB-910 (obtained from acutely HIV- 1 infected individual; SeraCare BioServices, Gaithersburg, MD) were used as bait for affinity selection of phages displaying HIV-1 peptides. After four rounds of biopanning, 22 clones (for each plasma sample) were selected for insert sequencing, and were analyzed by phage ELISA with HIV positive and negative sera, to confirm the specificity of reactivity. Alignment of inserts with the HIV-1 genome led to identification of twelve immunodominant epitopes, mapping to Gag-p24 & p6, Pol, Env-gp120 & gp41, and Nef. Interestingly, phages displaying sequences from the intracytoplasmic tail of gp41 (amino acids 784-871) were repeatedly recognized by antibodies from both early (1-6 months) and chronically infected individuals. The cytoplasmic tail of gp41 was selected as the primary candidate for the differential assay as it is unlikely to be targeted by HIV-neutralizing antibodies, and it is not included in most HIV vaccines currently under development. In addition, a p6 sequence was

also selected, even though it was included in early generation HIV vaccines, it contains very few HLA restricted CTL epitopes (Frahm, N. *et al.* (2004) "CONSISTENT CYTOTOXIC-T-LYMPHOCYTE TARGETING OF IMMUNODOMINANT REGIONS IN HUMAN IMMUNODEFICIENCY VIRUS ACROSS MULTIPLE ETHNICITIES. J Virol 78:2187-2200) (Los Alamos database; http://hiv-web.lanl.gov). Importantly, the selected gp41 [spanning amino acids 784-829 (SK1) and 836-871 (SK2)], and the p6 (amino acids 452-502) sequences are highly conserved among all HIV-1 M subtypes.

Establishment of the 'HIV-SELECTEST'

The p6 and two gp41-derived peptides were chemically synthesized and used for the development of the new assay. Based on the Los Alamos HIV sequence database, consensus peptides were designed to encompass the genetic variability among HIV-1 clades. Initially, each peptide was evaluated individually to determine specificity and establish cut-off values. Since both gp41 peptides (SK1 and SK2) displayed similar very low reactivity with HIV seronegative samples, the two were combined. Multiple ELISA conditions were tested and after screening of 1000 seronegative samples, cut-off (CO) values for the gp41 (CO=0.03) and p6 (CO=0.15) peptides were determined. Each CO value represents the average absorbance of negative sera + 5 standard deviations. Additional panels containing high, intermediate, and low HIV-specific antibody titers were used to determine the dynamic range of the assay. **Figure 7, Panels (a)** and **(b)** demonstrate the binding of serially diluted representative plasma, PRB-204-06 (from SeraCare BioServices), in the p6 and gp41 ELISA, respectively. Multiple titrations with different samples demonstrated higher maximum reactivity with the gp41 peptides and a broader dynamic range when compared with the p6 peptide. Based on these analyses, all subsequent ELISA testing was conducted with 1:100 dilution of sera or plasma. The HIV infection status of a given sample was determined by licensed detection kits conducted either in-house or by an outside laboratory. An assay specificity of 100% for the gp41 peptides and 99.4% for the p6 peptide was established after screening of > 2500 samples from uninfected or from individuals infected with diverse HIV-1 clades. The combined

sensitivity of the gp41 and p6 peptides is 99.3% for detection of early and chronic infections in multiple geographical sites with clades A, B, C, D, E, F, J and multiple circulating recombinant forms.

Assay Robustness And Statistical Analysis

The reproducibility of the assay was determined by repeatedly testing nine HIV seropositive and three HIV seronegative samples from SeraCare BioServices. The distributions of the results obtained on multiple dates were evaluated for normality and the appropriate p-values were calculated using SigmaPlot. Representative plots are shown for one individual on the p6 and gp41 peptides (**Figure 7**, **Panels (c) and (d)**, respectively. The upper and lower limits (± 2SD) represent the 95% confidence intervals. Inter-assay variability was ≤ 10% and intra-assay variability was ≤ 5% for all the samples tested.

Acute Infections Are Detected With HIV-SELECTEST

To determine how soon post-infection HIV-specific antibodies are detected with the HIV-SELECTEST, several well-characterized seroconversion panels were obtained from SeraCare BioServices containing sequential bleeds within 30-40 days of estimated exposure dates. As shown in **Table 12** top panel, the p6 peptide reacted positively with PBR-910 on collection day 26, in agreement with results obtained using licensed HIV antibody detection kits. The gp41 peptides were reactive with the day 32 sample from the same individual. For PRB-929, day 25 and day 28 samples reacted with p6 and gp41 peptides, respectively (**Table 13**). In that individual, infection was confirmed by PCR on day 14 and the Abbott HIV Ag test was positive on day 18. In **Tables 12** and **13**, ELISA data for P6 and GP41 are shown as the ratio of test specimen absorbance to cut-off value. Ratios of 1.00 or greater are considered HIV seropositive and a sample ratio of less than 1 is considered HIV negative; for Abbott assays, PCR, and and FDA licensed EIA kits, HIV early seroconversion panels (within 6 weeks after HIV infection) and data for HIV RNA PCR quantification and FDA licensed serodiagnostic kits were provided by SeraCare BioServices, (Gaithersburg, MD).

Similar results were obtained with additional seroconversion panels from SeraCare BioServices, and demonstrated that HIV infection could be detected by the HIV-SELECTEST within 2-4 weeks following HIV-1 RNA detection by PCR, concurrent with the sensitivity limits of licensed HIV diagnostic tests. In addition, we evaluated 28 seroconversion panels spanning 6-18 months post-infection from Australia (**Table 14**). With these panels, p6 showed variable reactivity at later times post-infection, whereas anti-gp41 reactivity increased over time and was maintained at high levels in most individuals, indicating that the kinetics and avidity of the antibody responses against the p6 and gp41 epitopes were not linked. In Table 23, the date of infection was estimated to be the midpoint between the last seronegative and first seropositive results obtained with licensed HIV diagnostic kits.

Evaluation of samples from HIV vaccine trials

The main proof-of-concept in support of the HIV-SELECTEST should come from evaluating the reactivity of vaccine induced antibodies in the course of prophylactic vaccine trials. To that end, six blinded panels from completed vaccine trials (502 vaccinees) were tested including HVTN 203 (conducted by the HIV Vaccine Trial Network), RV124 (conducted by the Walter Reed Army Institute of Research) and VRC 004, VRC 006, VRC 009, and VRC 010 (conducted by the Vaccine Research Center, NIAID, NIH). The description of the vaccine constructs used in the various trials and summary of the results obtained with the HIV-SELECTEST appear in **Table 22**. Canarypox vaccine constructs used in the RV124 and HVTN 203 contained the p6 epitope used in the new assay. Additionally, the protein boost in RV124 was gp160. In contrast, the vaccine constructs used in VRC 004 and VRC 006 lacked the peptide sequences used in the HIV-SELECTEST.

Table 22
Summary of HIV-SELECTEST Reactivity with Vaccine Trial Samples

Sponsor & Vaccine Trial Number	Vaccine Composition		Total No. of Samples Tested	No. of HIV Infected Samples	No. of Samples Positive by FDA Licensed Kits (%)	HIV-SELECTEST Reactivity	
	Prime	Booster				p6 (%)	gp41 (%)
RV124[1]	vCP205 (gag + pro+ env)	gp160 protein	79	0	63 (80%)	2 (2.5%)	0
HVTN 203[2]	vCP1452 (gag-pro-gp140 + Pol & Nef-CTL epitopes	gp140 protein	324	**2**	97 (30%)	38 (12%)	**2 (0.6%)**[5]
VRC 004[3]	pGag-Pol-Nef + pEnvA + pEnvB + pEnvC	SAME AS PRIME	50[6]	**2**	15 (38%)	**1 (2%)**[5]	**2 (4%)**[5]
VRC 006[3]	Ad5-Gag-Pol + Ad5-EnvA + Ad5-EnvB + Ad5-EnvC	-	36[6]	0	18 (60%)	0	0
VRC 009[3]	pGag-Pol-Nef + pEnvA + pEnvB + pEnvC	Ad5-Gag-Pol + Ad5-EnvA + Ad5-EnvB + Ad5-EnvC	9[6]	0	9 (100%)	0	0
VRC 010[3]	pGag + pPol + pNef + pEnvA + pEnvB + pEnvC	Ad5-Gag-Pol + Ad5-EnvA + Ad5-EnvB + Ad5-EnvC	4[6]	0	4 (100%)	0	0

1. The RV124 vaccine immunogens contained both the p6 & gp41 peptides used in the HIV-SELECTEST.
2. The HVTN 203 vaccine immunogen contained the p6 but not the gp41 sequences used in HIV-SELECTEST.
3. The VRC 004, 006, 009 vaccines did not contain either p6 or gp41 epitopes used in the HIV-SELECTEST, but participants in VRC 010 received p6-containing DNA (pGag) prime during VRC 007 phase I trial.
4. Bio-Rad HIV-1/2+O EIA kit was used for sample screening. Seroconversion in VRC 009 and VRC 010 was determined by rapid tests. Capillus HIV-1/HIV-2 and Uni-Gold HIV.
5. Upon unblinding, these seropositive samples were confirmed as true HIV infections.
6. VRC 004 had 10 placebo subjects and VRC 006 had 6 placebos, while VRC 009 and VRC 010 had no placebo subjects.

The RV124 trial represents the worst case scenario, wherein all the peptide sequences used in the HIV-SELECTEST were part of either the priming or boosting immunogens. After the last boost (day 182), 80% of vaccinees strongly seroconverted in commercial HIV-1 detection kits even though none were HIV infected (**Table 22** and **Table 20**). However, only two individuals scored positive in the p6-ELISA, and none reacted in the gp41-ELISA. These findings suggested that the epitopes used in the HIV-SELECTEST were not very immunogenic in the context of the RV124 vaccine constructs.

The HVTN 203 blinded specimens included samples from pre-vaccination and four & six months post-vaccination obtained from 324 trial participants. In this panel, 30% of vaccinees seroconverted in the licensed HIV detection assays, while only 12% reacted with the p6 peptide in the HIV-SELECTEST (**Table 22**). This finding was not surprising since the Canarypox/HIV prime (vCP1452) contained p6. Unexpectedly, two specimens were repeatedly reactive with gp41 even though these sequences were not in the vaccine constructs. However, after unblinding, it was confirmed that both samples were obtained from trial participants who got infected during this phase II trial.

The VRC phase I trials VRC 004, VRC 006, VRC 009, and VRC 010 were conducted in 2002-2005. The DNA plasmids (VRC 004) and non-replicating recombinant Adenovirus serotype 5 vector (rAd5) (VRC 006) express Gag-Pol-Nef (in VRC 004) or Gag-Pol (in VRC 006) and multi-clade (A, B, C) envelope genes (gp145 in the DNA vaccine and gp140 in the rAd5 vaccine). Among the 50 participants in VRC 004, 38% (15/40) of vaccinated individuals seroconverted by licensed HIV diagnostic kits (**Table 22**). Unexpectedly, two samples reacted positive in the gp41 ELISA, of which one sample also reacted with p6 in the HIV-SELECTEST (**Table 22**). Upon unblinding, it was determined that both individuals (both in the placebo arm) became infected during the VRC 004 trial. In the VRC 006 (Ad5/HIV), no intercurrent HIV infections were identified, yet 60% of vaccine recipients (18/30) tested positive in licensed HIV detection tests. In contrast, none of the vaccinees reacted with either the p6 or gp41 in the HIV-SELECTEST (**Table 22**). In VRC 009 and VRC 010, a subset of DNA vaccinated individuals (from VRC 004 and VRC 007 trials, respectively) was

boosted with the rAd5/HIV vaccine. The 4 weeks post-boost samples demonstrated a very significant increase in total HIV-specific antibodies (data not shown), and 100% seroconversion using two licensed rapid tests (Capillus HIV-1/HIV-2 and Uni-Gold HIV, Trinity Biotech, NY). Importantly, all vaccinees in these trials tested negative in the HIV-SELECTEST (**Table 22**).

Detection of intercurrent HIV Infections during Vaccine Trials

Data obtained with the blinded panels from HIV vaccine trials tested to date indicates that vaccine–generated antibodies are most likely to give negative reactivity in the HIV-SELECTEST, especially if the vaccines do not contain the p6 sequence. Importantly, the new test detected all intercurrent infections in the blinded samples. To further determine the sensitivity of new assay in detecting acute HIV infections in the course of vaccine trials, sequential samples were tested from HIV infections in completed Phase I, Phase II, and Phase III trials conducted by HVTN (Lee, D. *et al.* (2004) "BREAKTHROUGH INFECTIONS DURING PHASE 1 AND 2 PRIME-BOOST HIV-1 VACCINE TRIALS WITH CANARYPOX VECTORS (ALVAC) AND BOOSTER DOSE OF RECOMBINANT GP120 OR GP160," J Infect Dis 190:903-907) VRC, and VaxGen (VAX 003 / VAX 004 efficacy trials) (Gilbert, P.B. *et al.* (2005) "CORRELATION BETWEEN IMMUNOLOGIC RESPONSES TO A RECOMBINANT GLYCOPROTEIN 120 VACCINE AND INCIDENCE OF HIV-1 INFECTION IN A PHASE 3 HIV-1 PREVENTIVE VACCINE TRIAL," J Infect Dis 191:666-677 (2005).

As can be seen in **Table 23**, **Figure 6, Panel (a)**, and **Table 24,** sequential samples obtained from 22 vaccinees infected with HIV during the HVTN trials and the VRC 004 trial reacted positive in the HIV-SELECTEST at early time points after the estimated infection dates. Importantly, no reactivity in the HIV-SELECTEST was observed prior to HIV infections in trial participants, even though they were immunized with complex vaccine products.

Table 23
HIV-SELECTEST Specifically Detects Intercurrent HIV Infections During Multiple HIV Vaccine Trials

Serum Sample	Days After Estimated Date of HIV Infection [1]	HIV-SELECTEST		Vaccine Immunogen
		p6	gp41	
HIVNET-1-1	-102.00	0.52	0.13	vCP205 +SF-2 rgp120
HIVNET-1-2	71	0.71	**83.33**	vCP205 + SF-2 rgp120
HIVNET-1-3	99	**6.41**	**80.77**	vCP205 + SF-2 rgp120
HIVNET-2-1	-158.00	0.22	0.30	vCP205 + Saline placebo
HIVNET-2-2	24	**11.19**	0.13	vCP205 + Saline placebo
HIVNET-2-3	87	**11.74**	**88.83**	vCP205 + Saline placebo
HVTN 203-1-1	-22	0.32	0.13	vCP1452 mo(0,1,3,6) + AIDSVAX B/B mo(3,6)
HVTN 203-1-2	16	**15.78**	0.10	vCP1452 mo(0,1,3,6) + AIDSVAX B/B mo(3,6)
HVTN 203-1-3	712	**1.01**	**16.83**	vCP1452 mo(0,1,3,6) + AIDSVAX B/B mo(3,6)
AVEG-1-1	-9	0.67	0.10	vCP205 (mo 0,1) + SF-2 rpg120 (mo 6,9,12)
AVEG-1-2	31	**15.25**	**1.13**	vCP205 (mo 0,1) + SF-2 rpg120 (mo 6,9,12)
AVEG-1-3	72	**16.51**	**46.33**	vCP205 (mo 0,1) + SF-2 rpg120 (mo 6,9,12)
VRC 004-1-1	-9	0.06	0.07	Placebo
VRC 004-1-2	19	**1.15**	0.17	Placebo
VRC 004-1-3	47	**2.95**	0.33	Placebo
VRC 004-1-4	130	**9.97**	**23.07**	Placebo

1 Sequential samples from HIV infections during VRC 004 and multiple HVTN clinical trials and the estimated dates of infection were provided by the clinical oversight boards.

Table 24
Reactivity Of Intercurrent Hiv Infection Samples During HVTN & VRC-004 Clinical Trials In The 'HIV-SELECTEST'

Serum Sample	Days Post Infection	New HIV ELISA'		Vaccine Type
		p6	gp41	
HVTN-1-1	-106.00	0.27	0.07	vCP205 + rgp120
HVTN-1-2	71	**1.92**	**8.10**	
HVTN-1-3	105.00	**1.31**	**59.27**	
HVTN-2-1	-19.00	0.08	0.13	Placebo-Saline
HVTN-2-2	51	0.22	**3.13**	
HVTN-2-3	67	0.17	**33.00**	
HVTN-3-1	-67.00	0.04	0.13	Placebo-Saline
HVTN-3-2	58	0.40	**46.70**	
HVTN-3-3	79	0.28	**50.77**	
HVTN-4-1	-55.00	0.03	0.13	vCP205 + Saline
HVTN-4-2	72	**1.09**	0.13	
HVTN-4-3	85	**1.15**	0.17	
HVTN-5-1	-102.00	0.52	0.13	vCP205 + rgp120
HVTN-5-2	71	0.71	**83.33**	
HVTN-5-3	99	**6.41**	**80.77**	
HVTN-6-1	-30.00	0.07	0.17	vCP205 + rgp120
HVTN-6-2	11	0.04	0.07	
HVTN-6-3	53	0.78	**28.37**	
HVTN-7-1	-158.00	0.22	0.30	vCP205 + Saline
HVTN-7-2	24	**11.19**	0.13	
HVTN-7-3	87	**11.74**	**88.83**	
HVTN-8-1	-20.00	0.08	0.30	vCP205 + rgp120
HVTN-8-2	30	0.35	0.23	
HVTN-8-3	44	0.25	**5.07**	
HVTN-9-1	-91.00	0.78	0.17	vCP205 + Saline
HVTN-9-2	106	**4.22**	**14.33**	
HVTN-9-3	169.00	0.87	**4.37**	
HVTN-10-1	-52	0.49	0.10	vCP1452
HVTN-10-2	39	**6.29**	0.17	
HVTN-10-3	52	**5.16**	**2.33**	
HVTN-11-1	-14	0.09	0.10	vCP1452 + AIDSVAX B/B
HVTN-11-2	91	0.64	**78.07**	

Table 24
Reactivity Of Intercurrent Hiv Infection Samples During HVTN & VRC-004 Clinical Trials In The 'HIV-SELECTEST'

Serum Sample	Days Post Infection	New HIV ELISA'		Vaccine Type
		p6	gp41	
HVTN-12-1	-60	0.09	0.07	vCP1452
HVTN-12-2	38	0.45	**6.03**	
HVTN-13-1	-22	0.32	0.13	vCP1452 + AIDSVAX B/B
HVTN-13-2	16	**15.78**	0.10	
HVTN-13-3	712	**1.01**	**16.83**	
HVTN-14-1	-9	0.03	0.10	Placebo-Saline
HVTN-14-2	5	0.05	0.20	
HVTN-14-3	95	0.13	**23.30**	
HVTN-15-1	-9	0.67	0.10	vCP205 + rgp120
HVTN-15-2	31	**15.25**	**1.13**	
HVTN-15-3	72	**16.51**	**46.33**	
HVTN-16-1	-1030	0.39	0.13	vCP205 + gp160
HVTN-16-2	211	0.17	**87.17**	
HVTN-16-3	307	0.13	**92.33**	
HVTN-17-1	-39.00	0.02	0.10	Placebo-Saline
HVTN-17-2	37	0.11	**23.67**	
HVTN-17-3	66	0.15	**44.70**	
HVTN-18-1	-408.00	0.05	0.10	vCP65 + rgp120
HVTN-18-2	242.00	0.13	**82.20**	
HVTN-18-3	272.00	0.16	**85.63**	
HVTN-19-1	-90.00	0.37	0.13	vCP205 + rgp120
HVTN-19-2	92	**3.97**	**31.10**	
HVTN-19-3	191.00	**3.70**	**77.33**	
HVTN-20-1	-37.00	0.03	0.07	Placebo-Saline
HVTN-20-2	63	0.77	**42.83**	
VRC-004-1-1	-136	0.09	0.47	Placebo-Saline
VRC-004-1-2	-40	0.28	0.37	
VRC-004-1-3	44	0.61	**42.43**	
VRC-004-2-1	-13	0.05	0.07	Placebo-Saline
VRC-004-2-2	14	**1.15**	0.17	
VRC-004-2-3	69	**2.95**	0.33	
VRC-004-2-4	145	**9.97**	**23.07**	

Sequential samples soon after the first confirmed PCR positive visit were also obtained from 65 HIV infections during VAX 003 (AIDSVAX gp120 B/E)

and 81 HIV infections during VAX 004 (AIDSVAX gp120 B/B') trials conducted by VaxGen. The dates of PCR positivity and seroconversion by licensed HIV tests were provided by VaxGen. **Table 25** contains analysis of two representative HIV infections in VAX 003 and VAX 004 trials that developed strong reactivity to p6 and gp41 peptides. Furthermore, the HIV-SELECTEST identified all intercurrent HIV infections within 90 days of PCR confirmation (**Figure 6, Panels (b) and (c); Table 26** and **Table 27,** for VAX 003 and VAX 004, respectively).

Table 25
Early Diagnosis Of Intercurrent HIV Infections by HIV-SELECTEST During VaxGen Clinical Trials VAX 003 & VAX 004

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-9-1	02/02/00	+	-	0.69	0.23
VAX-003-9-2	16/02/00	+	+	**7.49**	**2.13**
VAX-003-9-3	09/03/00	+	+	**12.17**	**15.57**
VAX-003-9-4	20/03/00	+	+	**14.42**	**70.4**
VAX-003-9-5	18/04/00	+	+	**7.18**	**45.53**
VAX-003-14-1	01/02/01	+	-	**4.05**	0.23
VAX-003-14-2	15/02/01	+	-	**12.83**	0.9
VAX-003-14-3	15/03/01	+	+	**8.21**	**12.33**
VAX-003-14-4	12/04/01	+	+	**7.32**	**12.3**
VAX-003-14-5	10/05/01	+	+	**6.56**	**13.17**
VAX-004-9-1	17/02/99	+	-	0.03	0.11
VAX-004-9-2	02/03/99	+	-	0.31	0.15
VAX-004-9-3	22/03/99	+	-	**4.73**	**1.04**
VAX-004-9-4	06/04/99	+	+	**2.73**	**5.15**
VAX-004-51-1	14/12/98	+	-	0.51	0.19
VAX-004-51-2	28/12/98	+	-	**3.15**	0.3
VAX-004-51-3	12/01/99	+	-	**5.69**	**14.22**
VAX-004-51-4	27/01/99	+	+	**3.87**	**51.33**

1 Sequential samples from HIV infections during VAX 003 and VAX 004 clinical trials and the results of PCR and licensed HIV serodiagnostic assays were provided by the VaxGen clinical lab

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-1-1	7/15/1999	+	-	0.09	0.00
VAX-003-1-2	7/29/1999	+	-	0.06	0.17
VAX-003-1-3	12/9/1999	+	+	**1.47**	**59.60**
VAX-003-1-4	12/28/1999	+	+	**1.14**	**53.37**
VAX-003-1-5	1/4/2000	+	+	**1.01**	**50.20**
VAX-003-2-1	10/1/2001	+	-	0.07	**2.20**
VAX-003-2-2	10/15/2001	+	+	0.13	**21.20**
VAX-003-2-3	11/7/2001	+	+	0.38	**80.23**
VAX-003-2-4	12/4/2001	+	+	0.23	**87.30**
VAX-003-3-1	10/1/2001	+	-	0.58	0.20
VAX-003-3-2	10/15/2001	+	-	**1.77**	0.80
VAX-003-3-3	3/26/2002	+	+	0.47	**41.53**
VAX-003-3-4	4/9/2002	+	+	**4.31**	**81.17**
VAX-003-3-5	5/17/2002	+	+	**3.77**	**83.60**
VAX-003-4-1	1/13/2000	+	-	0.04	0.27
VAX-003-4-2	1/27/2000	+	-	0.05	0.23
VAX-003-4-3	6/26/2000	+	+	**1.17**	**76.80**
VAX-003-4-4	7/14/2000	+	+	0.79	**63.17**
VAX-003-4-5	7/24/2000	+	+	0.59	**69.27**
VAX-003-5-1	4/24/2002	+	-	0.07	0.20
VAX-003-5-2	5/8/2002	+	+	0.08	0.73
VAX-003-5-3	8/28/2002	+	+	0.07	**86.47**
VAX-003-5-4	12/11/2002	+	+	0.08	**82.63**
VAX-003-6-1	6/22/1999	+	-	0.10	0.23
VAX-003-6-2	7/6/1999	+	-	0.03	0.13
VAX-003-6-3	7/22/1999	+	+	0.21	**23.50**
VAX-003-6-4	8/9/1999	+	+	0.11	**89.90**
VAX-003-6-5	8/19/1999	+	+	0.18	**93.50**
VAX-003-7-1	1/9/2001	+	-	0.33	0.20
VAX-003-7-2	1/23/2001	+	-	**1.28**	0.73
VAX-003-7-3	6/26/2001	+	+	0.25	**39.43**
VAX-003-7-4	7/17/2001	+	+	0.33	**42.17**
VAX-003-7-5	7/27/2001	+	+	0.27	**39.83**
VAX-003-8-1	8/25/1999	+	-	0.19	0.10
VAX-003-8-2	9/8/1999	+	-	**12.41**	0.43
VAX-003-8-3	1/20/2000	+	+	0.53	**83.40**
VAX-003-8-4	6/13/2000	+	+	0.19	**83.97**
VAX-003-8-5	8/29/2000	+	+	0.32	**89.20**
VAX-003-9-1	2/2/2000	+	-	0.69	0.23
VAX-003-9-2	2/16/2000	+	+	**7.49**	**2.13**
VAX-003-9-3	3/9/2000	+	+	**12.17**	**15.57**
VAX-003-9-4	3/20/2000	+	+	**14.42**	**70.40**
VAX-003-9-5	4/18/2000	+	+	**7.18**	**45.53**
VAX-003-10-1	6/6/2000	+	-	0.07	0.23
VAX-003-10-2	6/20/2000	+	-	0.11	0.13
VAX-003-10-3	11/20/2000	+	+	0.20	**83.60**

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-10-4	12/18/2000	+	+	0.11	**77.57**
VAX-003-10-5	1/5/2001	+	+	0.11	**84.07**
VAX-003-11-1	8/1/2001	+	-	0.26	0.20
VAX-003-11-2	8/15/2001	+	-	0.33	0.17
VAX-003-11-3	1/16/2002	+	+	0.48	**2.87**
VAX-003-11-4	2/1/2002	+	+	**1.90**	**14.10**
VAX-003-11-5	2/15/2002	+	+	**1.57**	**11.93**
VAX-003-12-1	3/15/2000	+	-	0.07	0.13
VAX-003-12-2	3/28/2000	+	-	0.22	0.10
VAX-003-12-3	8/30/2000	+	+	0.55	**81.40**
VAX-003-12-4	9/11/2000	+	+	0.25	**70.73**
VAX-003-12-5	9/25/2000	+	+	0.41	**80.20**
VAX-003-13-1	8/5/1999	+	-	0.06	0.17
VAX-003-13-2	8/19/1999	+	-	0.07	0.17
VAX-003-13-3	9/2/1999	+	-	0.08	0.03
VAX-003-13-4	9/15/1999	+	-	0.33	**1.80**
VAX-003-13-5	10/6/1999	+	+	0.61	**18.77**
VAX-003-13-6	11/16/1999	+	+	**2.01**	**36.57**
VAX-003-13-7	11/30/1999	+	+	0.91	**35.37**
VAX-003-14-1	2/1/2001	+	-	**4.05**	0.23
VAX-003-14-2	2/15/2001	+	-	**12.83**	0.90
VAX-003-14-3	3/15/2001	+	+	**8.21**	**12.33**
VAX-003-14-4	4/12/2001	+	+	**7.32**	**12.30**
VAX-003-14-5	5/10/2001	+	+	**6.56**	**13.17**
VAX-003-15-1	2/21/2001	+	-	**1.25**	0.17
VAX-003-15-2	3/8/2001	+	-	**1.39**	0.20
VAX-003-15-3	8/29/2001	+	+	0.79	**88.90**
VAX-003-15-4	9/27/2001	+	+	0.71	**81.17**
VAX-003-15-5	10/24/2001	+	+	0.68	**80.80**
VAX-003-16-1	10/20/1999	+	-	0.23	0.13
VAX-003-16-2	11/3/1999	+	-	0.37	0.27
VAX-003-16-3	11/17/1999	+	-	0.29	**4.67**
VAX-003-16-4	12/1/1999	+	+	0.22	**30.80**
VAX-003-16-5	12/15/1999	+	+	0.16	**66.13**
VAX-003-16-6	1/6/2000	+	+	0.22	**82.23**
VAX-003-17-1	9/22/2000	+	-	0.09	0.27
VAX-003-17-2	10/6/2000	+	-	0.09	0.30
VAX-003-17-3	3/9/2001	+	+	0.36	**83.40**
VAX-003-17-4	3/23/2001	+	+	0.19	**84.27**
VAX-003-17-5	4/9/2001	+	+	0.23	**85.80**
VAX-003-18-1	8/9/1999	+	-	0.20	0.17
VAX-003-18-2	8/23/1999	+	-	**1.47**	0.63
VAX-003-18-3	9/6/1999	+	+	**5.07**	**25.90**
VAX-003-18-4	9/17/1999	+	+	**2.89**	**39.00**
VAX-003-18-5	10/1/1999	+	+	**2.39**	**69.73**
VAX-003-19-1	8/14/2001	+	-	0.16	0.13

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-19-2	9/3/2001	+	-	0.11	0.47
VAX-003-19-3	1/29/2002	+	+	0.27	**14.53**
VAX-003-19-4	2/20/2002	+	+	**1.17**	**51.33**
VAX-003-19-5	3/6/2002	+	+	0.83	**42.07**
VAX-003-20-1	1/8/2001	+	-	**1.37**	**25.63**
VAX-003-20-2	1/22/2001	+	+	**1.53**	**34.60**
VAX-003-20-3	2/5/2001	+	+	0.83	**28.33**
VAX-003-20-4	2/21/2001	+	+	0.79	**40.83**
VAX-003-21-1	11/16/1999	+	-	0.21	0.20
VAX-003-21-2	11/30/1999	+	-	0.16	0.13
VAX-003-21-3	12/14/1999	+	-	0.19	0.17
VAX-003-21-4	12/28/1999	+	+	0.24	**1.13**
VAX-003-21-5	1/11/2000	+	+	0.21	**11.53**
VAX-003-21-6	1/28/2000	+	+	0.50	**38.43**
VAX-003-22-1	11/10/1999	+	-	**1.91**	0.03
VAX-003-22-2	11/24/1999	+	-	**1.24**	0.10
VAX-003-22-3	4/21/2000	+	+	**2.28**	**85.60**
VAX-003-22-4	5/11/2000	+	+	**2.37**	**86.97**
VAX-003-22-5	11/2/2000	+	+	**1.25**	**85.40**
VAX-003-23-1	3/7/2002	+	-	0.31	0.10
VAX-003-23-2	3/21/2002	+	-	0.36	0.10
VAX-003-23-3	8/21/2002	+	+	0.41	**92.23**
VAX-003-23-4	9/11/2002	+	+	**1.97**	**93.60**
VAX-003-23-5	10/17/2002	+	+	**2.98**	**94.47**
VAX-003-24-1	1/19/2000	+	-	0.04	0.17
VAX-003-24-2	2/2/2000	+	-	0.05	0.07
VAX-003-24-3	2/16/2000	+	+	0.15	0.47
VAX-003-24-4	3/8/2000	+	+	0.30	**35.87**
VAX-003-24-5	3/15/2000	+	+	0.41	**65.67**
VAX-003-25-1	1/27/2000	+	-	0.12	0.17
VAX-003-25-2	2/10/2000	+	-	0.09	0.07
VAX-003-25-3	2/23/2000	+	+	0.05	0.33
VAX-003-25-4	3/13/2000	+	+	0.13	**13.23**
VAX-003-25-5	3/24/2000	+	+	0.12	**17.23**
VAX-003-26-1	2/1/2000	+	-	0.03	0.13
VAX-003-26-2	2/17/2000	+	-	0.37	0.10
VAX-003-26-3	6/22/2000	+	+	**1.33**	**80.00**
VAX-003-26-4	9/13/2000	+	+	0.45	**69.80**
VAX-003-26-5	11/6/2000	+	+	0.55	**85.30**
VAX-003-27-1	1/4/2000	+	-	**1.99**	0.27
VAX-003-27-2	1/17/2000	+	-	**7.77**	0.10
VAX-003-27-3	5/23/2000	+	+	**6.41**	**71.83**
VAX-003-27-4	6/12/2000	+	+	**3.79**	**57.77**
VAX-003-27-5	6/19/2000	+	+	**4.27**	**63.70**
VAX-003-28-1	5/11/2000	+	-	0.18	0.20
VAX-003-28-2	5/25/2000	+	-	0.23	0.10

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-28-3	10/25/2000	+	+	**1.76**	**91.73**
VAX-003-28-4	11/28/2000	+	+	0.95	**90.67**
VAX-003-28-5	12/20/2000	+	+	**1.42**	**94.77**
VAX-003-29-1	6/13/2000	+	-	0.05	0.10
VAX-003-29-2	8/3/2000	+	-	0.30	**87.20**
VAX-003-29-3	11/23/2000	+	+	0.20	**92.07**
VAX-003-29-4	12/22/2000	+	+	0.16	**97.23**
VAX-003-29-5	1/8/2001	+	+	0.05	**95.70**
VAX-003-30-1	12/22/1999	+	-	0.04	0.10
VAX-003-30-2	1/5/2000	+	-	0.04	0.17
VAX-003-30-3	1/18/2000	+	+	0.11	0.50
VAX-003-30-4	1/31/2000	+	+	0.13	**16.50**
VAX-003-30-5	2/29/2000	+	+	0.09	**36.33**
VAX-003-31-1	12/29/1999	+	-	**2.77**	0.33
VAX-003-31-2	1/12/2000	+	+	**3.89**	0.50
VAX-003-31-3	3/29/2000	+	+	**7.35**	**8.90**
VAX-003-31-4	5/12/2000	+	+	**4.45**	**12.83**
VAX-003-32-1	3/7/2000	+	-	**1.83**	0.10
VAX-003-32-2	3/21/2000	+	-	**2.19**	0.07
VAX-003-32-3	7/25/2000	+	+	**2.17**	**85.57**
VAX-003-32-4	8/11/2000	+	+	0.72	**81.80**
VAX-003-32-5	8/30/2000	+	+	0.95	**81.17**
VAX-003-33-1	7/25/2000	+	-	0.19	**6.70**
VAX-003-33-2	8/8/2000	+	+	0.23	**12.43**
VAX-003-33-3	8/18/2000	+	+	0.09	**5.17**
VAX-003-33-4	9/8/2000	+	+	0.10	**3.93**
VAX-003-34-1	10/16/2001	+	-	0.05	0.17
VAX-003-34-2	10/30/2001	+	-	0.04	0.17
VAX-003-34-3	4/23/2002	+	+	0.04	**77.67**
VAX-003-34-4	5/14/2002	+	+	0.15	**91.90**
VAX-003-34-5	5/23/2002	+	+	0.18	**91.90**
VAX-003-35-1	1/17/2000	+	-	0.80	-0.03
VAX-003-35-2	1/31/2000	+	-	**3.61**	0.70
VAX-003-35-3	2/14/2000	+	+	**6.53**	**17.03**
VAX-003-35-4	3/3/2000	+	+	**2.87**	**49.10**
VAX-003-35-5	3/20/2000	+	+	**2.45**	**77.63**
VAX-003-36-1	1/21/2000	+	-	0.83	0.13
VAX-003-36-2	2/7/2000	+	-	0.75	0.20
VAX-003-36-3	2/16/2000	+	-	0.56	0.47
VAX-003-36-4	3/1/2000	+	-	0.78	**12.83**
VAX-003-36-5	4/5/2000	+	+	0.74	**18.40**
VAX-003-36-6	4/24/2000	+	+	0.67	**29.90**
VAX-003-36-7	5/12/2000	+	+	0.50	**27.30**
VAX-003-37-1	1/24/2001	+	-	0.08	0.10
VAX-003-37-2	2/7/2001	+	-	0.63	**1.13**
VAX-003-37-3	7/11/2001	+	+	0.61	**1.63**

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-37-4	8/3/2001	+	+	0.75	**2.10**
VAX-003-37-5	8/17/2001	+	+	0.67	**4.47**
VAX-003-38-1	12/15/2000	+	-	0.06	0.13
VAX-003-38-2	12/28/2000	+	-	0.07	0.17
VAX-003-38-3	5/18/2001	+	+	0.03	**31.40**
VAX-003-38-4	6/1/2001	+	+	0.05	**45.17**
VAX-003-38-5	6/13/2001	+	+	0.05	**54.13**
VAX-003-39-1	1/17/2000	+	-	0.49	0.27
VAX-003-39-2	1/31/2000	+	-	0.64	0.10
VAX-003-39-3	2/14/2000	+	+	0.89	0.17
VAX-003-39-4	3/6/2000	+	+	0.55	**9.70**
VAX-003-39-5	3/16/2000	+	+	0.52	**20.07**
VAX-003-40-1	6/19/2001	+	-	0.07	0.47
VAX-003-40-2	8/6/2001	+	+	0.09	**9.77**
VAX-003-40-3	8/27/2001	+	+	0.11	**30.83**
VAX-003-40-4	9/14/2001	+	+	0.05	**40.50**
VAX-003-41-1	11/29/2001	+	-	0.07	0.13
VAX-003-41-2	12/13/2001	+	-	0.09	0.13
VAX-003-41-3	3/11/2002	+	+	**6.83**	**79.80**
VAX-003-41-4	3/26/2002	+	+	**9.95**	**90.63**
VAX-003-41-5	4/9/2002	+	+	**8.75**	**92.20**
VAX-003-42-1	1/19/2000	+	-	0.09	0.20
VAX-003-42-2	2/2/2000	+	+	**1.38**	0.77
VAX-003-42-3	2/17/2000	+	+	0.69	**13.77**
VAX-003-42-4	3/23/2000	+	+	0.87	**22.23**
VAX-003-43-1	1/15/2002	+	-	0.05	0.13
VAX-003-43-2	1/31/2002	+	-	0.06	0.20
VAX-003-43-3	7/15/2002	+	+	0.09	**63.87**
VAX-003-43-4	8/7/2002	+	+	0.17	**76.53**
VAX-003-43-5	8/21/2002	+	+	0.15	**81.37**
VAX-003-44-1	4/5/2000	+	-	0.05	0.17
VAX-003-44-2	4/19/2000	+	-	0.07	0.17
VAX-003-44-3	9/7/2000	+	+	**2.33**	**58.07**
VAX-003-44-4	10/16/2000	+	+	**1.18**	**39.40**
VAX-003-44-5	11/2/2000	+	+	0.73	**34.37**
VAX-003-45-1	3/7/2002	+	-	0.28	0.37
VAX-003-45-2	3/20/2002	+	-	0.22	0.23
VAX-003-45-3	8/6/2002	+	+	0.21	**78.80**
VAX-003-45-4	8/30/2002	+	+	0.35	**86.13**
VAX-003-45-5	9/10/2002	+	+	0.28	**79.07**
VAX-003-46-1	8/23/2001	+	-	**2.05**	**49.57**
VAX-003-46-2	9/6/2001	+	-	**1.01**	**48.90**
VAX-003-46-3	2/7/2002	+	+	0.71	**16.03**
VAX-003-46-4	3/14/2002	+	+	**1.06**	**76.90**
VAX-003-46-5	5/30/2002	+	+	0.83	**78.97**
VAX-003-47-1	5/9/2000	+	-	0.99	0.33

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-47-2	5/25/2000	+	-	0.79	0.07
VAX-003-47-3	6/8/2000	+	+	**1.01**	**1.43**
VAX-003-47-4	8/2/2000	+	+	0.29	**63.67**
VAX-003-47-5	9/29/2000	+	+	0.26	**72.13**
VAX-003-48-1	4/26/2000	+	-	**1.33**	0.63
VAX-003-48-2	5/10/2000	+	+	**1.07**	**11.27**
VAX-003-48-3	5/25/2000	+	+	0.40	**52.40**
VAX-003-48-4	6/23/2000	+	+	0.33	**61.27**
VAX-003-49-1	4/24/2001	+	-	0.06	0.10
VAX-003-49-2	5/9/2001	+	-	0.05	0.07
VAX-003-49-3	9/20/2001	+	+	0.95	**89.80**
VAX-003-49-4	10/4/2001	+	+	0.99	**86.63**
VAX-003-49-5	10/26/2001	+	+	**1.30**	**89.77**
VAX-003-50-1	4/1/2002	+	-	0.07	0.10
VAX-003-50-2	4/17/2002	+	-	0.08	0.23
VAX-003-50-3	8/30/2002	+	+	0.09	**78.00**
VAX-003-50-4	9/20/2002	+	+	0.13	**81.07**
VAX-003-50-5	11/4/2002	+	+	0.14	**82.93**
VAX-003-51-1	3/4/2002	+	-	**2.40**	0.13
VAX-003-51-2	3/21/2002	+	-	**1.99**	0.10
VAX-003-51-3	8/20/2002	+	+	0.27	**71.43**
VAX-003-52-1	9/12/2000	+	-	0.27	**1.07**
VAX-003-52-2	9/26/2000	+	+	0.31	**6.53**
VAX-003-52-3	10/11/2000	+	+	0.07	**20.03**
VAX-003-52-4	10/26/2000	+	+	0.13	**10.07**
VAX-003-53-1	6/15/2000	+	-	0.10	0.13
VAX-003-53-2	6/28/2000	+	-	0.17	0.07
VAX-003-53-3	11/1/2000	+	+	0.41	**10.53**
VAX-003-53-4	11/15/2000	+	+	0.23	**7.20**
VAX-003-53-5	11/28/2000	+	+	0.17	**6.00**
VAX-003-54-1	6/12/2000	+	-	**1.93**	0.07
VAX-003-54-2	6/26/2000	+	-	**3.84**	0.10
VAX-003-54-3	7/11/2000	+	+	**3.41**	**2.20**
VAX-003-54-4	7/26/2000	+	+	0.99	**3.87**
VAX-003-54-5	8/8/2000	+	+	**2.02**	**20.30**
VAX-003-55-1	10/3/2001	+	-	0.09	0.17
VAX-003-55-2	10/17/2001	+	-	0.09	0.03
VAX-003-55-3	3/18/2002	+	+	0.37	**27.47**
VAX-003-55-4	4/10/2002	+	+	0.84	**64.53**
VAX-003-55-5	4/24/2002	+	+	0.61	**57.60**
VAX-003-56-1	6/12/2000	+	-	0.08	0.27
VAX-003-56-2	7/20/2000	+	-	0.24	**10.27**
VAX-003-56-3	11/15/2000	+	+	0.11	**78.27**
VAX-003-56-4	12/19/2000	+	+	0.07	**71.00**
VAX-003-56-5	1/12/2001	+	+	0.05	**76.80**
VAX-003-57-1	6/29/2000	+	-	0.05	0.30

Table 26
Reactivity of Intercurrent HIV Infections During VAX-003 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-003-57-2	7/13/2000	+	-	0.07	0.20
VAX-003-57-3	11/16/2000	+	+	0.69	**53.63**
VAX-003-57-4	12/14/2000	+	+	0.32	**22.80**
VAX-003-57-5	12/21/2000	+	+	0.36	**22.37**
VAX-003-58-1	5/24/2000	+	-	0.13	0.17
VAX-003-58-2	6/7/2000	+	-	0.32	0.23
VAX-003-58-3	6/23/2000	+	+	0.43	**9.57**
VAX-003-58-4	7/7/2000	+	+	**1.70**	**37.17**
VAX-003-58-5	7/20/2000	+	+	**4.71**	**73.57**
VAX-003-59-1	4/17/2002	+	-	0.05	0.17
VAX-003-59-2	5/1/2002	+	-	0.06	0.13
VAX-003-59-3	10/1/2002	+	+	0.26	**49.53**
VAX-003-59-4	10/18/2002	+	+	0.85	**81.63**
VAX-003-60-1	8/30/2002	+	-	0.05	0.20
VAX-003-60-2	9/13/2002	+	-	0.07	0.27
VAX-003-60-3	4/4/2003	+	+	0.68	**50.27**
VAX-003-61-1	5/15/2001	+	-	0.07	0.27
VAX-003-61-2	5/30/2001	+	-	0.07	0.27
VAX-003-61-3	10/31/2001	+	+	0.12	**57.97**
VAX-003-61-4	11/14/2001	+	+	0.21	**82.40**
VAX-003-61-5	11/29/2001	+	+	0.29	**84.47**
VAX-003-62-1	11/21/2002	+	-	0.05	0.17
VAX-003-62-2	12/3/2002	+	-	0.07	0.20
VAX-003-62-3	5/7/2003	+	+	0.37	**89.37**
VAX-003-63-1	9/20/2000	+	-	0.33	0.13
VAX-003-63-2	10/4/2000	+	-	0.43	0.13
VAX-003-63-3	2/13/2001	+	+	**3.66**	**86.27**
VAX-003-63-4	2/28/2001	+	+	**2.82**	**83.40**
VAX-003-63-5	3/13/2001	+	+	**3.07**	**86.03**
VAX-003-64-1	7/5/2000	+	-	0.06	0.10
VAX-003-64-2	7/19/2000	+	-	0.10	0.17
VAX-003-64-3	8/4/2000	+	+	0.13	0.87
VAX-003-64-4	8/25/2000	+	+	0.11	**64.30**
VAX-003-64-5	9/6/2000	+	+	0.06	**66.97**
VAX-003-65-1	9/21/2000	+	-	0.16	0.10
VAX-003-65-2	10/5/2000	+	-	0.15	0.10
VAX-003-65-3	2/21/2001	+	+	0.49	**26.87**
VAX-003-65-4	3/15/2001	+	+	**1.53**	**38.03**
VAX-003-65-5	3/28/2001	+	+	**1.43**	**50.53**

Table 27
Reactivity of Intercurrent HIV Infections During VAX-004 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-004-1-1	2/11/2000	+	-	0.03	0.15
VAX-004-1-2	3/3/2000	+	-	0.46	**1.16**
VAX-004-1-3	4/3/2000	+	+	0.48	**60.36**
VAX-004-2-1	2/14/2001	+	-	0.50	0.37
VAX-004-2-2	3/1/2001	+	+	0.82	**1.84**
VAX-004-3-1	5/10/2000	+	-	0.07	0.11
VAX-004-3-2	5/25/2000	+	-	0.05	0.20
VAX-004-3-3	11/8/2000	+	+	0.25	**103.48**
VAX-004-4-1	3/20/2001	+	-	0.03	0.30
VAX-004-4-2	4/2/2001	+	-	0.07	**1.15**
VAX-004-4-3	10/3/2001	+	+	0.05	**99.60**
VAX-004-5-1	2/17/1999	+	-	0.07	0.19
VAX-004-5-2	3/3/1999	+	-	0.09	0.37
VAX-004-5-3	3/17/1999	+	-	0.09	0.11
VAX-004-5-4	3/31/1999	+	-	0.13	0.30
VAX-004-5-5	8/4/1999	+	+	0.25	**76.67**
VAX-004-6-1	9/21/2000	+	-	0.03	0.20
VAX-004-6-2	10/3/2000	+	-	0.33	0.15
VAX-004-6-3	10/18/2000	+	+	0.92	**3.48**
VAX-004-7-1	6/14/2000	+	-	0.31	**39.44**
VAX-004-7-2	12/18/2000	+	+	0.44	**74.07**
VAX-004-8-1	5/1/2001	+	-	0.12	0.15
VAX-004-8-2	8/8/2001	+	+	0.63	**99.37**
VAX-004-9-1	2/17/1999	+	-	0.03	0.11
VAX-004-9-2	3/2/1999	+	-	0.31	0.15
VAX-004-9-3	3/22/1999	+	-	**4.73**	**1.04**
VAX-004-9-4	4/6/1999	+	+	**2.73**	**5.15**
VAX-004-10-1	10/13/2000	+	-	0.17	**12.56**
VAX-004-10-2	4/11/2001	+	+	0.07	**16.56**
VAX-004-11-1	10/23/1998	+	-	0.19	0.44
VAX-004-11-2	11/3/1998	+	-	0.23	**1.33**
VAX-004-11-3	11/18/1998	+	+	0.18	**4.37**
VAX-004-12-1	11/2/2000	+	-	0.09	**1.37**
VAX-004-13-1	2/11/2000	+	-	0.06	0.11
VAX-004-13-2	8/10/2000	+	+	0.05	**87.08**
VAX-004-14-1	9/13/2000	+	-	**1.97**	**1.63**
VAX-004-14-2	9/27/2000	+	+	**1.81**	**38.40**
VAX-004-15-1	1/25/2001	+	-	0.03	0.11
VAX-004-15-2	2/8/2001	+	-	0.12	0.22
VAX-004-15-3	7/11/2001	+	+	0.02	**8.72**
VAX-004-16-1	2/22/2001	+	-	0.59	0.19
VAX-004-16-2	3/8/2001	+	-	0.45	0.19
VAX-004-16-3	8/29/2001	+	+	**1.00**	**82.28**
VAX-004-17-1	7/23/1999	+	-	0.15	0.19
VAX-004-17-2	8/6/1999	+	-	0.11	0.26
VAX-004-17-3	8/20/1999	+	+	0.27	**2.19**

Table 27
Reactivity of Intercurrent HIV Infections During VAX-004 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-004-18-1	2/10/2000	+	-	0.06	0.26
VAX-004-18-2	7/27/2000	+	+	0.09	**58.72**
VAX-004-19-1	12/5/2000	+	-	0.16	**8.33**
VAX-004-19-2	4/9/2001	+	+	0.65	**59.85**
VAX-004-20-1	3/6/2000	+	-	**2.13**	0.11
VAX-004-20-2	3/22/2000	+	-	**2.56**	0.33
VAX-004-20-3	10/3/2000	+	+	**1.43**	**106.70**
VAX-004-21-1	4/11/2001	+	-	0.01	0.04
VAX-004-21-2	6/14/2001	+	+	0.14	**21.93**
VAX-004-22-1	5/4/2000	+	-	0.37	0.22
VAX-004-22-2	9/12/2000	+	+	**1.15**	**96.70**
VAX-004-23-1	10/31/2000	+	-	0.20	0.96
VAX-004-23-2	11/15/2000	+	+	0.20	**13.12**
VAX-004-24-1	10/6/1999	+	-	0.03	0.11
VAX-004-24-2	10/21/1999	+	+	**3.92**	0.60
VAX-004-25-1	9/21/2000	+	-	0.14	0.22
VAX-004-25-2	9/26/2000	+	-	0.13	0.37
VAX-004-25-3	10/16/2000	+	+	**1.05**	**21.56**
VAX-004-26-1	1/6/1999	+	-	0.02	0.15
VAX-004-26-2	1/22/1999	+	-	0.13	0.22
VAX-004-26-3	5/28/1999	+	+	0.63	**18.44**
VAX-004-27-1	9/21/1999	+	-	0.23	0.15
VAX-004-27-2	10/14/1999	+	-	0.85	0.96
VAX-004-27-3	3/30/2000	+	+	**1.39**	**35.00**
VAX-004-28-1	1/14/2000	+	-	0.07	0.11
VAX-004-28-2	1/28/2000	+	-	0.07	0.11
VAX-004-28-3	7/11/2000	+	+	0.31	**87.04**
VAX-004-29-1	11/26/2001	+	-	0.19	0.20
VAX-004-29-2	3/18/2002	+	+	**2.09**	**95.56**
VAX-004-29-3	4/8/2002	+	+	**1.54**	**101.07**
VAX-004-30-1	2/28/2001	+	-	0.05	0.26
VAX-004-30-2	10/3/2001	+	+	0.08	**79.24**
VAX-004-31-1	10/20/2000	+	-	0.11	0.22
VAX-004-31-2	11/6/2000	+	-	0.11	**2.33**
VAX-004-31-3	6/12/2001	+	+	0.21	**111.12**
VAX-004-32-1	10/11/1999	+	-	0.10	0.19
VAX-004-32-2	10/25/1999	+	-	0.11	0.11
VAX-004-32-3	3/27/2000	+	+	0.81	**52.40**
VAX-004-33-1	6/15/2000	+	-	0.21	0.11
VAX-004-33-2	9/29/2000	+	+	**4.34**	**106.40**
VAX-004-34-1	1/23/2001	+	-	0.40	**3.04**
VAX-004-34-2	1/30/2001	+	+	0.49	**5.89**
VAX-004-35-1	10/27/2000	+	-	0.05	0.07
VAX-004-35-2	11/3/2000	+	-	0.12	0.15
VAX-004-35-3	11/16/2000	+	-	0.13	0.52

Table 27
Reactivity of Intercurrent HIV Infections During VAX-004 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-004-35-4	12/7/2000	+	+	0.05	**2.19**
VAX-004-36-1	7/24/2000	+	-	**1.91**	0.00
VAX-004-36-2	7/31/2000	+	+	**4.55**	0.24
VAX-004-37-1	4/14/1999	-	-	0.17	0.04
VAX-004-37-2	7/14/1999	+	-	**5.73**	0.07
VAX-004-37-3	7/21/1999	+	+	**8.76**	0.74
VAX-004-38-1	10/4/2000	+	-	0.03	0.11
VAX-004-38-2	10/19/2000	+	-	0.11	0.22
VAX-004-38-3	5/10/2001	+	+	**1.52**	**88.93**
VAX-004-39-1	5/19/1999	+	-	0.04	0.15
VAX-004-39-2	6/3/1999	+	-	0.13	0.19
VAX-004-39-3	6/17/1999	+	+	0.15	**1.10**
VAX-004-40-1	7/30/1999	+	-	0.06	0.11
VAX-004-40-2	8/12/1999	+	-	0.07	0.22
VAX-004-40-3	12/30/1999	+	+	0.77	**16.96**
VAX-004-41-1	6/11/2001	+	-	0.05	0.26
VAX-004-41-2	6/25/2001	+	+	**1.24**	0.28
VAX-004-42-1	6/13/2000	+	-	0.04	0.11
VAX-004-42-2	7/20/2000	+	+	0.24	**41.00**
VAX-004-43-1	8/27/1999	+	-	0.21	0.22
VAX-004-43-2	9/10/1999	+	-	0.50	0.26
VAX-004-43-3	3/10/2000	+	+	0.18	**30.44**
VAX-004-44-1	5/14/2001	+	-	0.15	0.33
VAX-004-44-2	8/24/2001	+	+	0.84	**27.78**
VAX-004-44-3	9/7/2001	+	+	0.61	**24.04**
VAX-004-45-1	7/31/2000	+	-	0.04	0.22
VAX-004-45-2	8/10/2000	+	-	0.07	0.22
VAX-004-45-3	2/19/2001	+	+	0.07	**10.16**
VAX-004-46-1	10/4/1999	+	-	**1.63**	0.11
VAX-004-46-2	10/18/1999	+	+	**1.60**	**2.04**
VAX-004-46-3	10/27/1999	+	+	**1.49**	**4.56**
VAX-004-47-1	8/2/2000	+	-	0.97	0.19
VAX-004-47-2	1/19/2001	+	-	**5.58**	**18.04**
VAX-004-47-3	2/8/2001	+	+	**5.55**	**14.80**
VAX-004-48-1	9/7/1999	+	-	0.15	0.15
VAX-004-48-2	9/27/1999	+	-	0.14	0.48
VAX-004-48-3	2/8/2000	+	+	0.16	**37.04**
VAX-004-49-1	11/14/2000	+	-	0.09	0.20
VAX-004-49-2	12/5/2000	+	+	**3.11**	0.78
VAX-004-50-1	11/7/2001	+	-	0.08	0.22
VAX-004-50-2	11/21/2001	+	-	0.73	0.52
VAX-004-50-3	12/18/2001	+	+	**1.67**	**88.28**
VAX-004-51-1	12/14/1998	+	-	0.51	0.19
VAX-004-51-2	12/28/1998	+	-	**3.15**	0.30
VAX-004-51-3	1/12/1999	+	-	**5.69**	**14.22**
VAX-004-51-4	1/27/1999	+	+	**3.87**	**51.33**

Table 27
Reactivity of Intercurrent HIV Infections During VAX-004 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-004-52-1	4/2/1999	+	-	0.26	**2.04**
VAX-004-52-2	4/16/1999	+	-	0.23	**5.41**
VAX-004-52-3	4/30/1999	+	+	0.55	**15.15**
VAX-004-53-1	11/30/2000	+	-	0.06	0.19
VAX-004-53-2	12/14/2000	+	-	0.07	0.15
VAX-004-53-3	5/23/2001	+	+	0.16	**102.52**
VAX-004-54-1	3/30/1999	+	-	0.44	0.22
VAX-004-54-2	4/7/1999	+	-	**1.36**	0.48
VAX-004-54-3	4/21/1999	+	+	**2.35**	**6.24**
VAX-004-55-1	9/14/1999	+	-	0.65	0.74
VAX-004-55-2	10/4/1999	+	+	**2.07**	**4.44**
VAX-004-56-1	3/19/2001	+	-	0.05	0.19
VAX-004-56-2	8/15/2001	+	+	**8.01**	0.40
VAX-004-57-1	1/24/2002	+	-	0.09	0.30
VAX-004-57-2	2/7/2002	+	+	0.19	**3.44**
VAX-004-57-3	2/11/2002	+	+	0.19	**3.93**
VAX-004-58-1	2/9/2000	+	-	0.15	0.32
VAX-004-58-2	8/8/2000	+	+	0.19	**38.12**
VAX-004-59-1	10/17/2001	+	-	0.04	0.15
VAX-004-59-2	11/9/2001	+	-	0.13	0.07
VAX-004-59-3	3/14/2002	+	+	0.24	**84.33**
VAX-004-60-1	7/5/2001	+	-	0.07	0.30
VAX-004-60-2	7/19/2001	+	-	0.04	**2.33**
VAX-004-60-3	11/9/2001	+	+	0.05	**9.67**
VAX-004-61-1	6/28/1999	+	-	0.47	0.59
VAX-004-61-2	7/8/1999	+	+	0.35	**1.20**
VAX-004-62-1	2/23/2000	+	-	0.90	0.11
VAX-004-62-2	3/7/2000	+	-	0.55	0.19
VAX-004-62-3	8/24/2000	+	+	0.81	**1.08**
VAX-004-63-1	9/20/1999	+	-	0.07	0.19
VAX-004-63-2	10/7/1999	+	-	0.17	0.56
VAX-004-63-3	10/21/1999	+	+	0.26	**3.84**
VAX-004-64-1	6/5/2001	+	-	0.03	0.11
VAX-004-64-2	10/23/2001	+	+	0.25	**101.26**
VAX-004-65-1	10/8/1999	+	-	0.05	0.22
VAX-004-65-2	10/26/1999	+	-	0.03	0.11
VAX-004-65-3	2/25/2000	+	+	0.11	**1.48**
VAX-004-66-1	9/3/1999	+	-	0.05	0.22
VAX-004-66-2	10/14/1999	+	-	0.04	**1.22**
VAX-004-66-3	11/17/1999	+	+	0.16	**9.00**
VAX-004-67-1	7/8/1999	+	-	0.65	0.30
VAX-004-67-2	7/20/1999	+	+	0.51	**1.03**
VAX-004-67-3	8/10/1999	+	+	**1.10**	**21.59**
VAX-004-68-1	9/4/2001	+	-	0.04	0.22
VAX-004-68-2	1/29/2002	+	+	0.09	**79.80**

Table 27
Reactivity of Intercurrent HIV Infections During VAX-004 Clinical Trials in the HIV-SELECTEST

Serum Sample	Draw Date	HIV PCR Analysis [1]	HIV Seroconversion (EIA and WB)	HIV-SELECTEST	
				p6	gp41
VAX-004-69-1	4/7/1999	+	-	0.20	0.11
VAX-004-69-2	4/21/1999	+	-	**5.29**	**10.22**
VAX-004-69-3	5/5/1999	+	+	**4.98**	**48.76**
VAX-004-70-1	12/13/1999	+	-	0.06	0.22
VAX-004-70-2	1/4/2000	+	-	0.11	0.22
VAX-004-70-3	2/29/2000	+	+	0.15	**14.92**
VAX-004-71-1	10/10/2001	+	-	0.41	0.19
VAX-004-71-2	10/25/2001	+	-	0.41	0.15
VAX-004-71-3	3/20/2002	+	+	**1.13**	**94.68**
VAX-004-72-1	11/6/2001	+	-	0.05	0.30
VAX-004-72-2	4/4/2002	+	+	0.09	**103.44**
VAX-004-73-1	2/23/2001	+	-	0.03	0.15
VAX-004-73-2	4/10/2001	+	+	0.49	**20.00**
VAX-004-74-1	12/9/1999	+	-	0.05	0.15
VAX-004-74-2	1/11/2000	+	+	0.07	**4.84**
VAX-004-75-1	8/2/2000	+	-	0.04	0.59
VAX-004-75-2	3/22/2001	+	+	0.05	**26.48**
VAX-004-76-1	2/26/2002	+	-	**1.28**	0.44
VAX-004-76-2	5/20/2002	+	+	**2.04**	**80.84**
VAX-004-77-1	5/10/2001	+	-	0.07	0.07
VAX-004-77-2	7/31/2001	+	+	0.23	**116.12**
VAX-004-78-1	10/14/1999	+	-	0.11	**1.00**
VAX-004-78-2	11/24/1999	+	+	**1.43**	**2.12**
VAX-004-79-1	11/3/1999	+	-	0.27	0.19
VAX-004-79-2	11/17/1999	+	-	0.36	**4.85**
VAX-004-79-3	3/23/2000	+	+	0.16	**1.08**
VAX-004-80-1	12/5/2000	+	-	0.07	0.26
VAX-004-80-2	4/23/2001	+	+	**1.85**	**48.00**
VAX-004-81-1	2/24/1999	+	-	0.23	0.19
VAX-004-81-2	3/11/1999	+	-	0.25	0.22
VAX-004-81-3	3/23/1999	+	-	0.17	0.41
VAX-004-81-4	4/6/1999	+	-	0.65	**4.15**
VAX-004-81-5	8/25/1999	+	+	0.24	**94.26**

It was also possible to compare the performance of the HIV-SELECTEST with results obtained with the FDA-licensed kits provided by VaxGen (**Figure 7**). In most cases, the earliest positive results were observed with the same samples using the licensed diagnostics and the HIV-SELECTEST (dots falling on the diagonal lines). Surprisingly, 24 intercurrent HIV infections in VAX 003, and 25 infections in VAX 004 were detected earlier in the HIV-SELECTEST compared with the licensed kits [dots under the diagonal lines in **Figure 7**, **Panels (a) and**

(b)], displaying the efficacy of HIV-SELECTEST in early diagnosis of HIV infection. Therefore, the new assay could be part of an algorithm that will provide an important differential diagnostic tool during future phase III prophylactic vaccine trials and for testing of blood and tissue donors.

The use of a phage display library to clone and express the entire open reading frames of HIV afforded the opportunity to identify all the epitopes that are recognized by seroconversion antibodies during acute HIV infection. Affinity selection of the phage display library using seroconversion panels led to the discovery of new epitopes in gp41 and p6, which were selected to develop a new differential diagnostic test.

The results described above demonstrate that vaccine generated antibodies scored either negative or weakly positive in the HIV-SELECTEST even when the p6 or gp41 sequences were part of the vaccine constructs (i.e., RV124 and HVTN 203). Furthermore, the HIV-SELECTEST detected all intercurrent HIV infections. It should be noted that while all intercurrent infections in the VAX 004 trial (conducted in the United States and the Netherlands) were with clade B viruses, all the HIV infections in the VAX 003 trial (conducted in Thailand), were with clade E variants, demonstrating the feasibility of using the HIV-SELECTEST outside the United States in a multiclade scenario, which is a prerequisite for global vaccine trials. Together, these data provide strong proof of the specificity and sensitivity of the new p6 and gp41 peptide-based ELISA. They further suggest that if future vaccine candidates do not contain these epitopes all uninfected vaccinees are expected to score negative in the new assay. In contrast, antibodies generated following intercurrent infections in the course of HIV vaccine trials, or at later times, should be detected by the HIV-SELECTEST soon after infection.

This inexpensive and high-throughput assay could be added to the algorithm of detection tests used in clinical sites and in blood and plasma collection centers. As such, this assay will be highly relevant for early diagnosis of intercurrent HIV infections in future vaccine trials, particularly in the setting of HIV vaccines that, while not able to prevent infection, may reduce viral loads after

acquisition. Importantly, the HIV-SELECTEST should help to alleviate the concerns regarding social and economic harms due to long-term seroconversion of uninfected participants in preventive HIV vaccine trials.

Example 10
Improved Gag-p6 peptide for HIV-1 Detection Assay

During the above-described testing, it was observed that some of the sera from individuals infected with clade C viruses (mainly from Southern Africa) did not react strongly with the consensus Gag-p6 employed (**SEQ ID NO:3**). The employed peptide was therefore altered to produce a peptide denoted "Gag-p6-C-sub" that is better recognized by all plasma and serum samples from HIV-1 clade C infected individuals. The sequence of the Gag-p6-C-sub peptide is provided below:

GAG-p6-C-sub (SEQ ID NO:141)

```
SRPEPTAPPA  ESFRFEETTP  APKQEPKDRE  PLTSLKSLFG  SDPLSQ
1          10          20          30          40      46
```

While HIV detection assays may be conducted using the Gag-p6-C-sub (**SEQ ID NO:141**) peptide alone, or in conjunction with any other peptide(s), etc., it is particularly preferred to combine the Gag p6-C-sub peptide (**SEQ ID NO:141**) with peptides having the sequence of the consensus Gag-p6 (**SEQ ID NO:3**) in the same ELISA plate.

All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS COMPREND PLUS D'UN TOME.

CECI EST LE TOME __1___ DE __2___

NOTE: Pour les tomes additionels, veillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

NOTE: For additional volumes please contact the Canadian Patent Office.

What Is Claimed Is:

Claim 1. A method for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of:

(a) contacting said biological sample with a peptide having an epitope that is recognized by said anti-HIV-1 antibody, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody if present in said sample to bind to said epitope and form a peptide-anti-HIV-1 antibody complex;

(b) contacting said formed peptide-anti-HIV-1 antibody complex with an anti-HIV-1 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody binding molecule to bind to anti-HIV-1 antibody of said formed peptide-anti-HIV-1 antibody complex and form an extended complex; and

(c) determining the presence or concentration of said anti-HIV-1 antibody in said biological sample by determining the presence or concentration of said formed extended complex;

wherein said epitope is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, 90** and **141.**

Claim 2. The method of claim 1, wherein said immunoassay is an ELISA.

Claim 3. The method of claim 2, wherein said ELISA comprises incubating said biological sample in the presence of a solid support, wherein said peptide is immobilized to said support, wherein said support is coated with milk, and wherein said anti-HIV-1 antibody binding molecule is selected from the group consisting of anti-human IgG + IgM-Fc, anti-human IgG-Fc, anti-human-IgG+IgM, and anti-human IgG; said anti-HIV-1 antibody binding molecule being conjugated to an enzyme.

Claim 4. The method of claim 1, wherein said immunoassay is an immunochromatographic assay.

Claim 5. The immunoassay of claim 4, wherein in said immunochromatographic immunoassay:

in said step (a), said biological sample is placed in contact with a first porous carrier, said first porous carrier containing said peptide, said peptide being non-immobilized and detectably labeled;

in said step (b), said formed peptide-anti-HIV-1 antibody complex is placed in contact with a second porous carrier, said second porous carrier being in communication with said first porous carrier, and containing an immobilized anti-HIV-1 antibody binding molecule; and

in said step (c), the presence or concentration of said anti-HIV antibody in said biological sample is determined by detecting the presence of said labeled peptide in said second porous carrier.

Claim 6. The method of claim 1, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:2**, **SEQ ID NO:3** or **SEQ ID NO:141**.

Claim 7. The method of claim 6, wherein said epitope is present on a peptide or protein having the amino acid sequence **of SEQ ID NO:2**.

Claim 8. The method of claim 6, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:3** or **SEQ ID NO:141**.

Claim 9. The method of claim 1, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:50, SEQ ID NO:51,** or **SEQ ID No. 55**.

Claim 10. The method of claim 6, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:50**.

Claim 11. The method of claim 6, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:51**.

Claim 12. The method of claim 6, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:55**.

Claim 13. A peptide or protein comprising an epitope that is recognized by an anti-HIV-1 antibody, wherein said epitope is present on a peptide having the amino acid sequence of **SEQ ID NO:3**, **SEQ ID NO:50,** or **SEQ ID NO:55**.

Claim 14. A peptide or protein having the amino acid sequence of **SEQ ID NO:3, SEQ ID NO:50, SEQ ID NO:55**, or **SEQ ID NO:141**.

Claim 15. An immunological complex comprising a peptide bound to an anti-HIV-1 antibody, wherein said anti-HIV-1 antibody is additionally bound to an anti-HIV antibody binding molecule, wherein said peptide or protein comprises an epitope that is recognized by an anti-HIV-1 antibody, said epitope being present on a peptide or protein having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, 90** and **141**.

Claim 16. A kit for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said kit comprises a hollow casing comprising a multilayer filter system, and first and second porous carriers, wherein said second porous carrier is in communication with said first porous carrier, and said first porous carrier is in communication with said multilayer filter system, a portion of which is accessible from said casing; wherein:

said first porous carrier contains a non-immobilized, labeled peptide or protein; and

said second porous carrier contains an immobilized, unlabeled antibody that binds to human IgG;

wherein said peptide or protein comprises an epitope that is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:1-11, 49-56, and 90**.

Claim 17. The kit of claim 16, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:2**, **SEQ ID NO:3** or **SEQ ID NO:141**.

Claim 18. The kit of claim 16, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:50, SEQ ID NO:51, or SEQ ID NO:55**.

Claim 19. The kit of claim 16, wherein said epitope is present on a peptide or protein having the amino acid sequence of **SEQ ID NO:90**.

Claim 20. A method for detecting the presence, or measuring the concentration, of an anti-HIV-2 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of:

(a) contacting said biological sample with a peptide having an epitope that is recognized by said anti-HIV-2 antibody, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody if present in said sample to bind to said epitope and form a peptide-anti-HIV-2 antibody complex;

(b) contacting said formed peptide-anti-HIV-2 antibody complex with an anti-HIV-2 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody binding molecule to bind to anti-HIV-2 antibody of said formed peptide-anti-HIV-2 antibody complex and form an extended complex; and

(c) determining the presence or concentration of said anti-HIV-2 antibody in said biological sample by determining the presence or concentration of said formed extended complex;

wherein said epitope is present on a peptide having an amino acid sequence selected from the group consisting of **SEQ ID NOs:101-102.**

Claim 21. A method for detecting the presence, or measuring the concentration, of an anti-HIV-1 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of:

(a) contacting said biological sample with an epitope set comprising at least one epitope that is recognized by said anti-HIV-1 antibody, wherein said epitope set consists essentially of an HIV-1 GAG p6 epitope or epitopes, an HIV-1 gp41 terminal region epitope or epitopes, or a combination of an HIV-1 GAG p6 epitope or epitopes and an HIV-1 gp41 terminal region epitope or epitopes, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody if present in said sample to bind to epitopes in said epitope set and form an epitope-anti-HIV-1 antibody complex;

(b) contacting said formed epitope-anti-HIV-1 antibody complex with an anti-HIV-1 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-1 antibody binding molecule to bind to anti-HIV-1 antibody of said formed epitope-anti-HIV-1 antibody complex and form an extended complex; and

(c) determining the presence or concentration of said anti-HIV-1 antibody in said biological sample by determining the presence or concentration of said formed extended complex.

Claim 22. The method of claim21, wherein said peptide set consists essentially of a combination of an HIV-1 GAG p6 epitope or epitopes and an HIV-1 gp41 terminal region epitope or epitopes.

Claim 23. A method for detecting the presence, or measuring the concentration, of an anti-HIV-2 antibody in a biological sample of a human, wherein said method comprises conducting an immunoassay comprising the steps of:

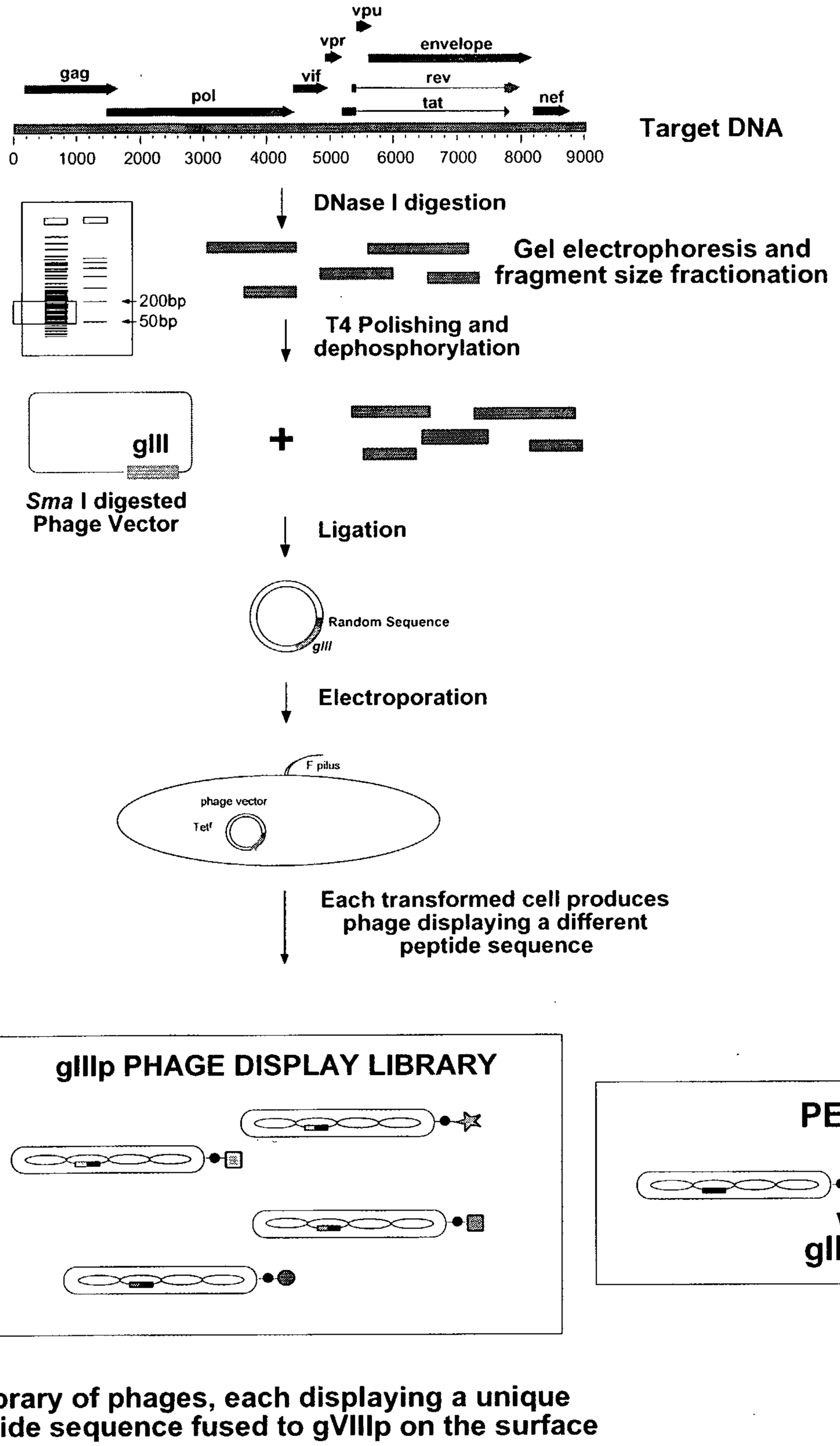
(a) contacting said biological sample with an epitope set comprising at least one epitope that is recognized by said anti-HIV-2 antibody, wherein said epitope set consists essentially of an HIV-2 GAG p6 epitope or epitopes, an HIV-2 Env-gp36 epitope or epitopes, or a combination of an HIV-2 GAG p6 epitope or epitopes and an HIV-2 Env-gp36 epitope or epitopes, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody if present in said sample to bind to epitopes in said epitope set and form an epitope-anti-HIV-2 antibody complex;

(b) contacting said formed epitope-anti-HIV-2 antibody complex with an anti-HIV-2 antibody binding molecule, said contacting being under conditions sufficient to permit said anti-HIV-2 antibody binding molecule to bind to anti-HIV-1 antibody of said formed epitope-anti-HIV-1 antibody complex and form an extended complex; and

(c) determining the presence or concentration of said anti-HIV-2 antibody in said biological sample by determining the presence or concentration of said formed extended complex.

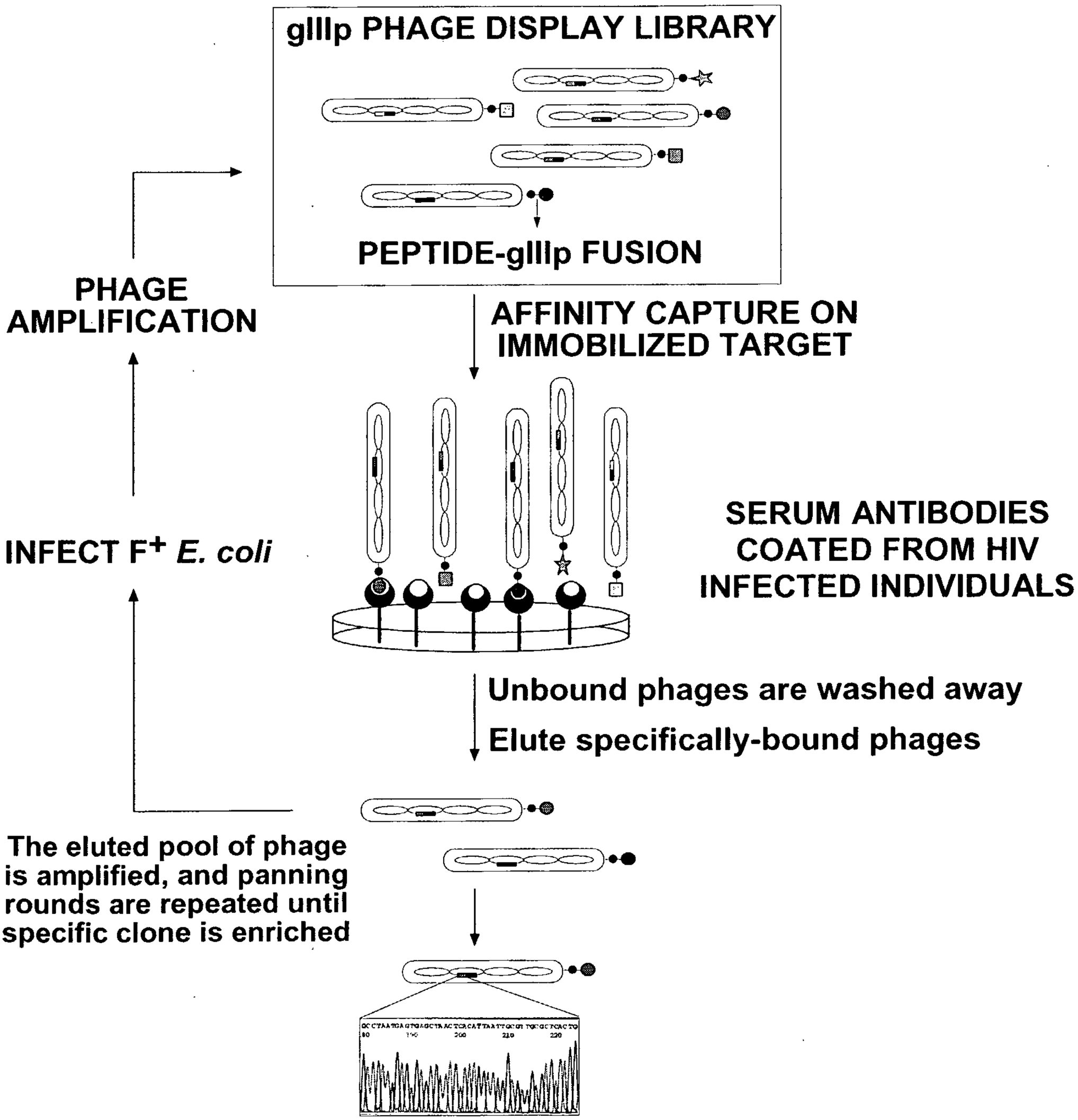
Claim 24. The method of claim23, wherein said peptide set consists essentially of a combination of an HIV-2 GAG-p6 epitope or epitopes and an HIV-2 Env-gp36 epitope or epitopes.

Figure 1



Library of phages, each displaying a unique peptide sequence fused to gVIIIp on the surface

Figure 2



ISOLATE SPECIFIC PHAGE AND SEQUENCE

Figure 3

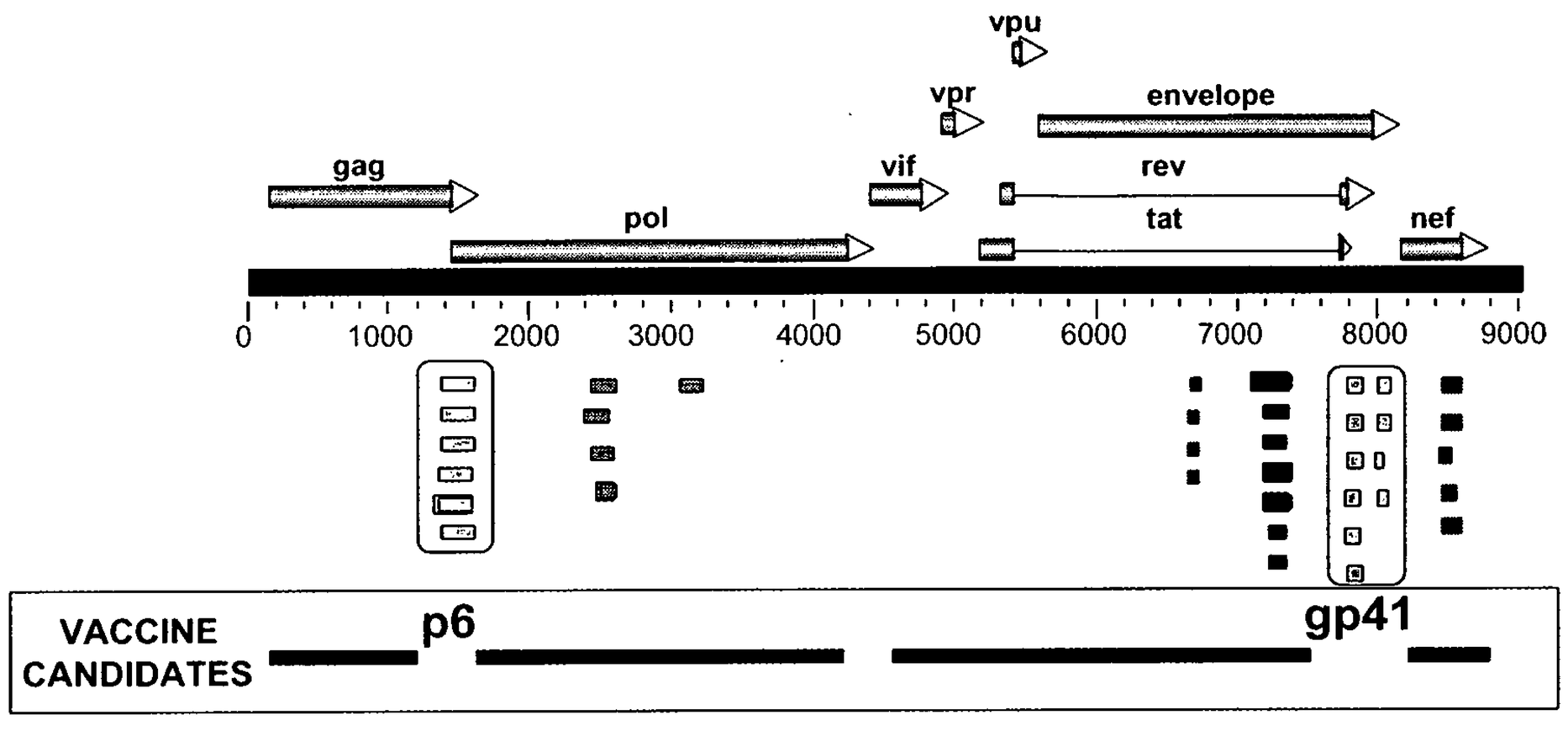
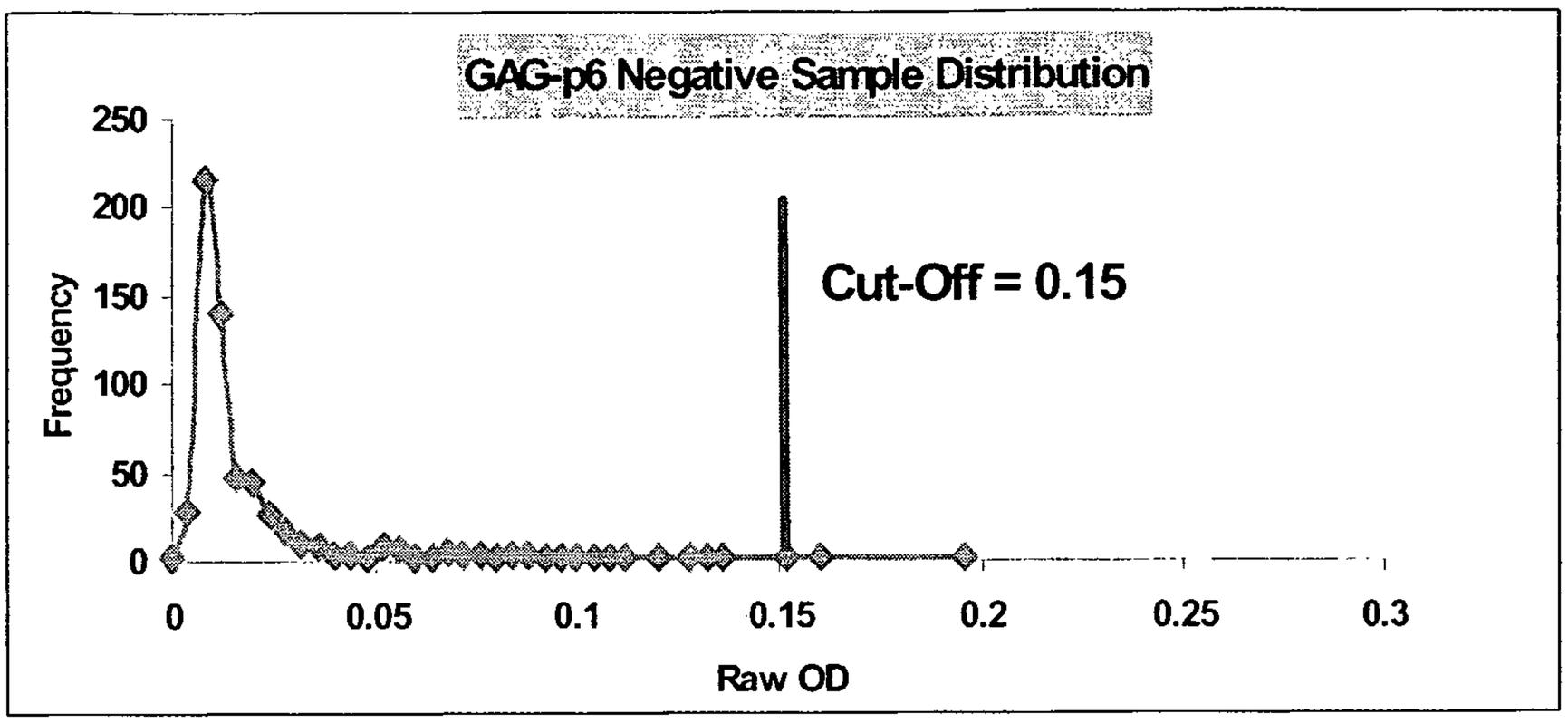

vpu
vpr
envelope
gag
vif
rev
pol
tat
nef
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
VACCINE CANDIDATES
p6
gp41

Figure 4

A


GAG-p6 Negative Sample Distribution
Frequency
250
200
150
100
50
0
Cut-Off = 0.15
0
0.05
0.1
0.15
0.2
0.25
0.3
Raw OD

B

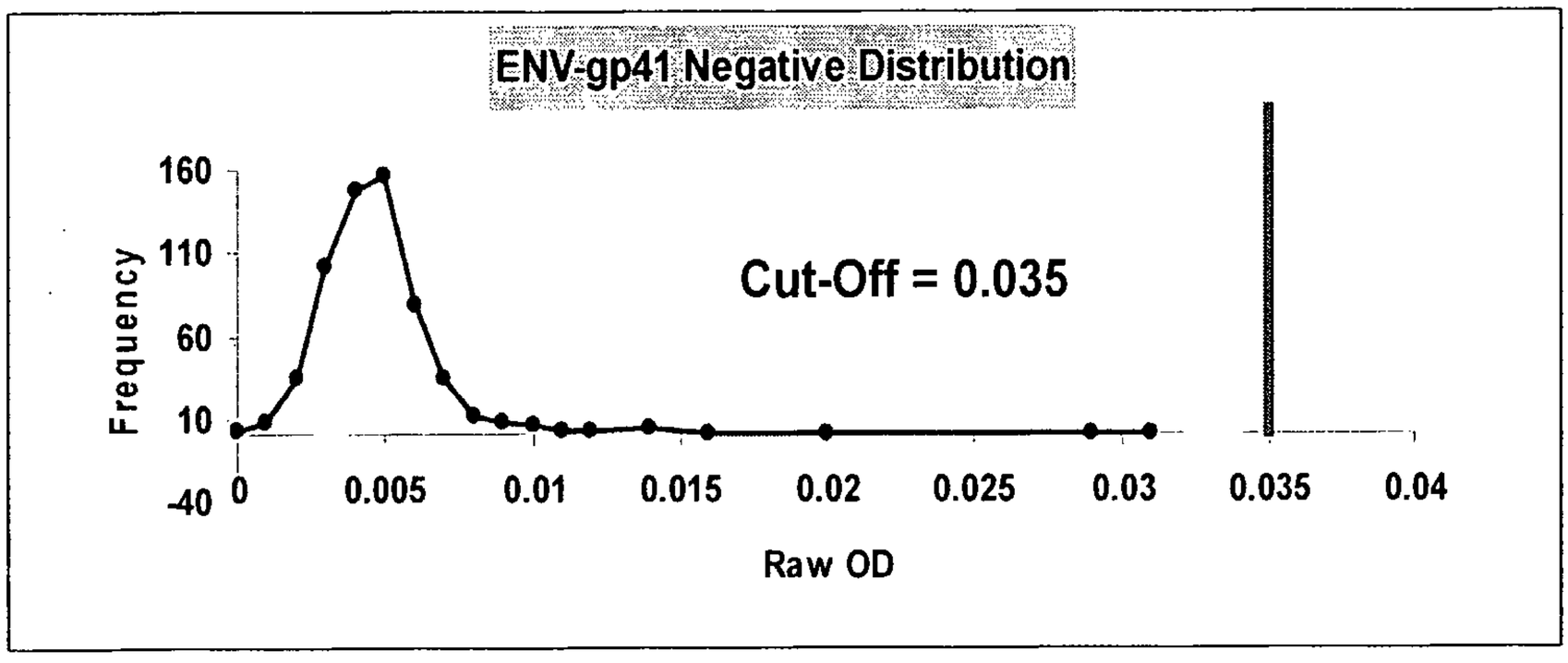
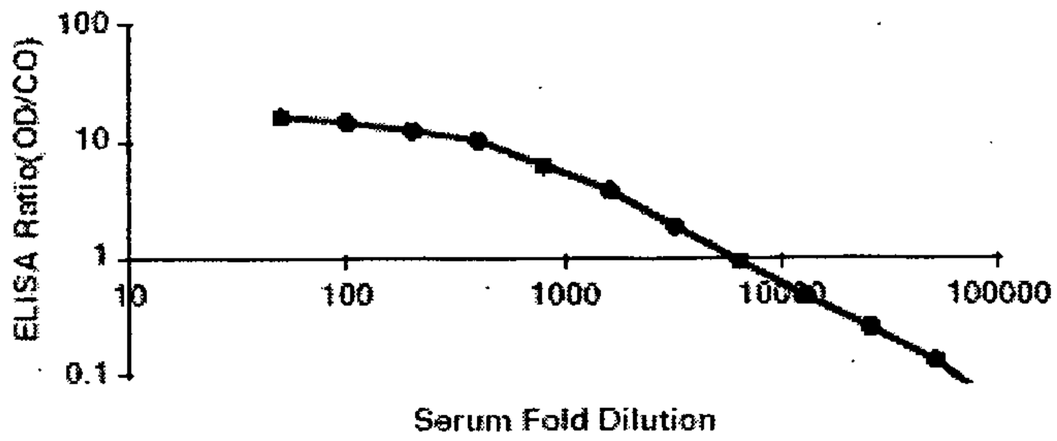

ENV-gp41 Negative Distribution
Frequency
160
110
60
10
-40
Cut-Off = 0.035
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
0.04
Raw OD

Figure 5

(a) p6



(b) gp41

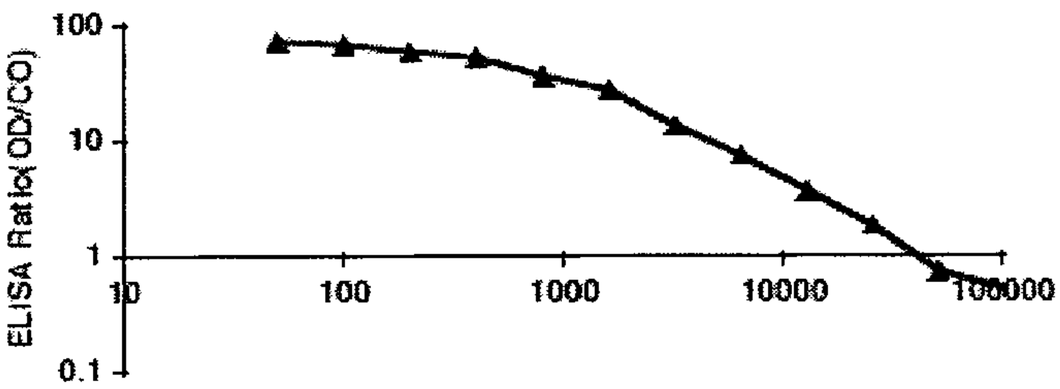
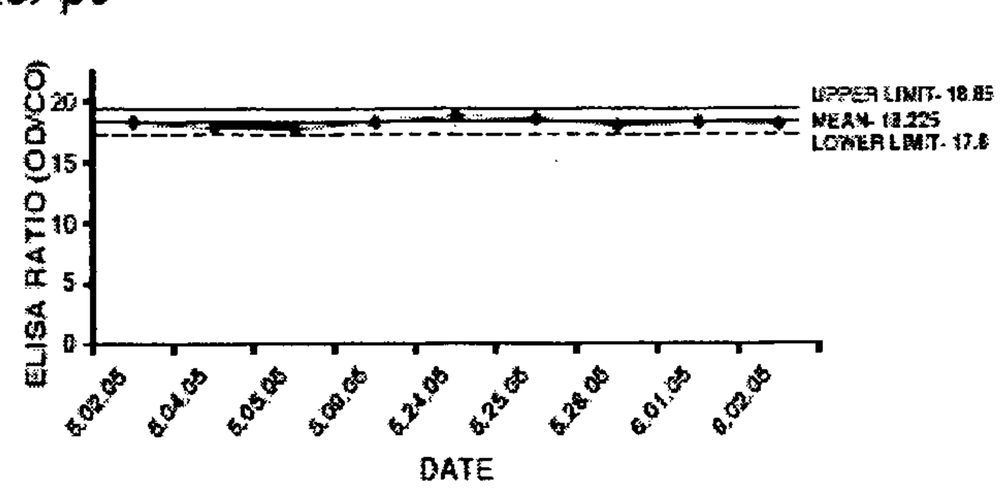


Figure 5 (cont.)

(c) p6



(d) gp41

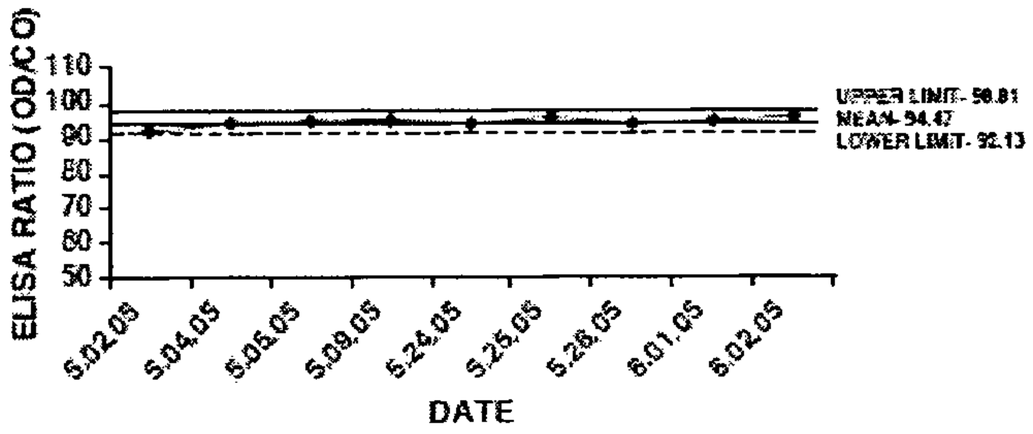
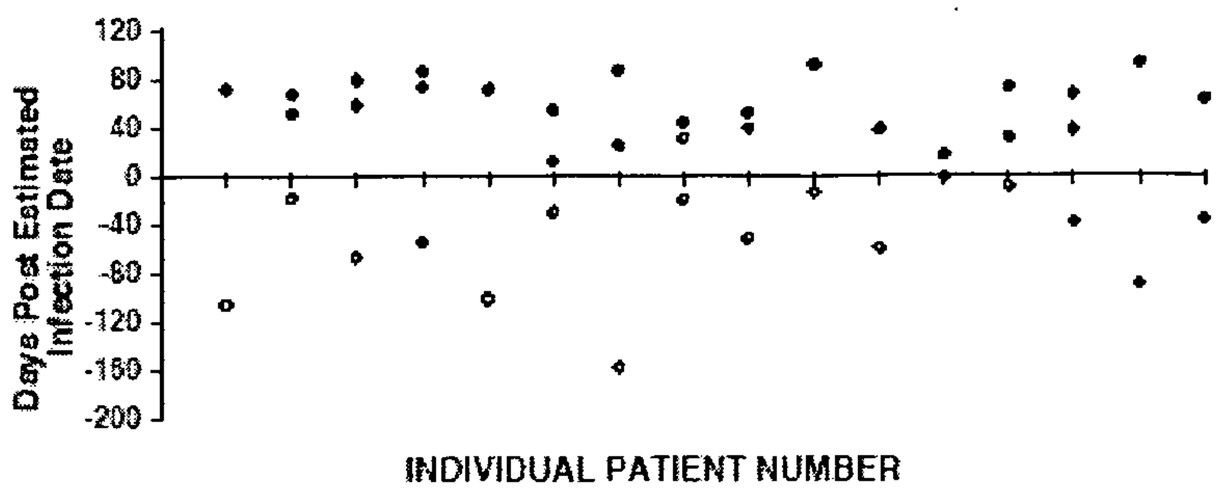
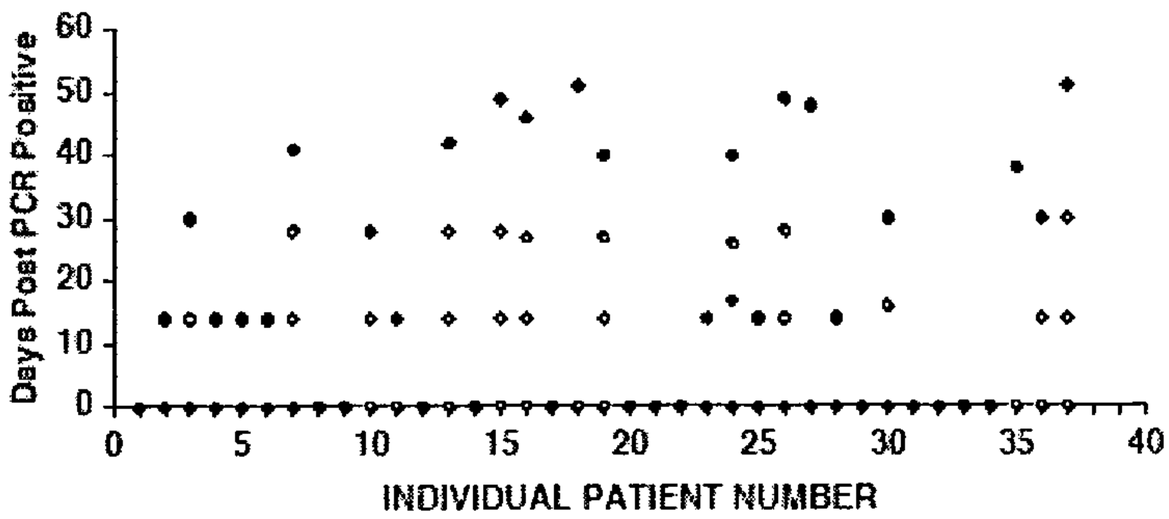


Figure 6

(a) MULTIPLE TRIALS



(b) VAX-003



(c) VAX-004

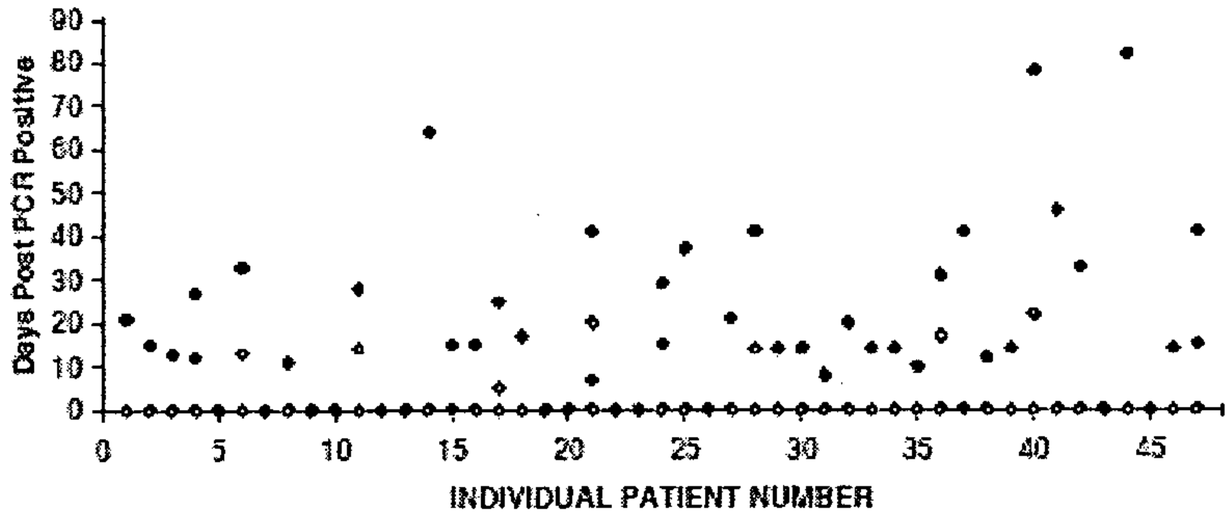
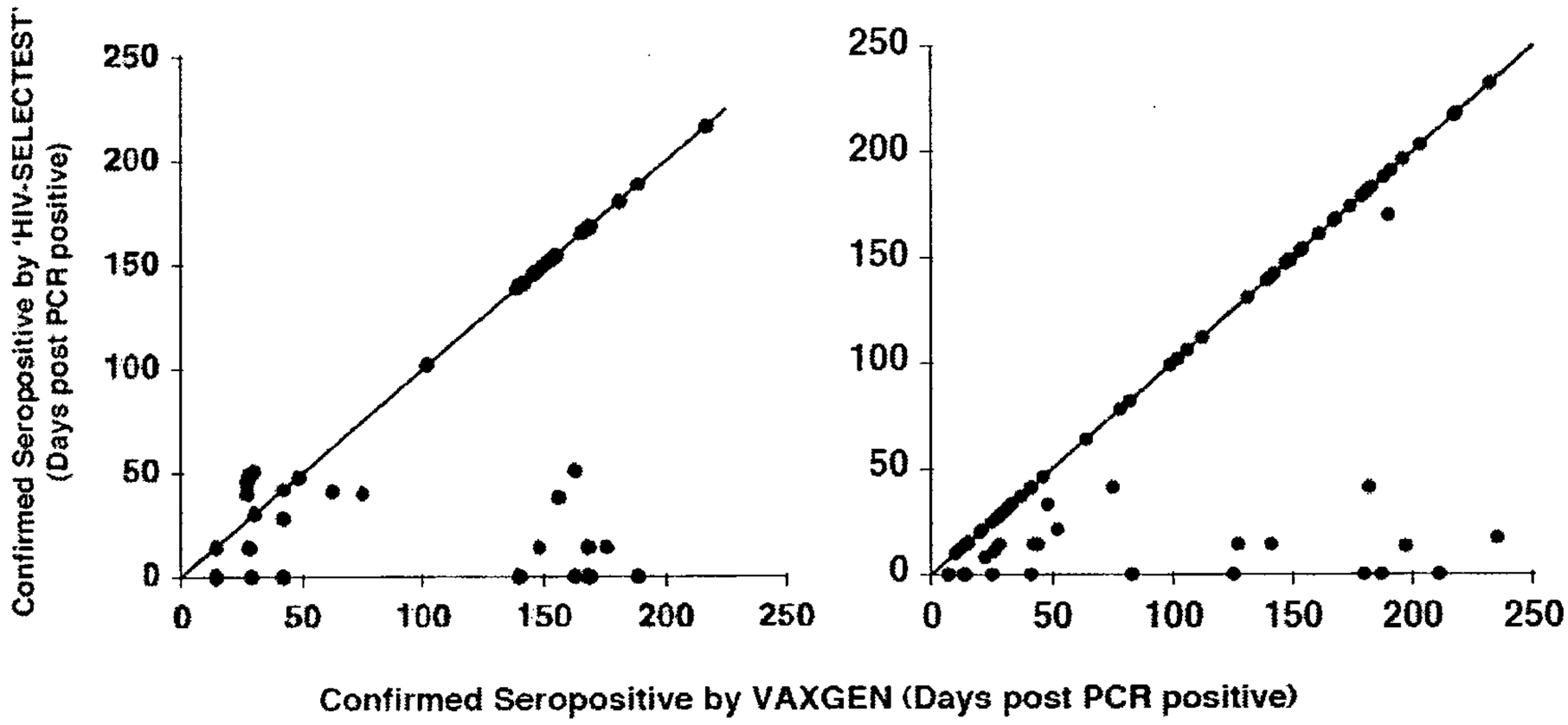


Figure 7


(a) VAX-003
(b) VAX-004
Confirmed Seropositive by 'HIV-SELECTEST' (Days post PCR positive)
0
50
100
150
200
250
Confirmed Seropositive by VAXGEN (Days post PCR positive)