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| <b>(54) Title:</b> IMMUNOFLUORESCENCE ASSAY FOR THE DETECTION OF ANTIBODIES USING RECOMBINANT ANTIGENS IN<br>INSOLUBLE FORM<br><br><b>(57) Abstract</b><br><br>The present invention relates to the use of insoluble forms of recombinant proteins in a flow cytometric immunofluorescence assay<br>for the detection of given antibodies.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |           |                                                                                                                                                                                                                                                                       |

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**IMMUNOFLUORESCENCE ASSAY  
FOR THE DETECTION OF ANTIBODIES USING  
RECOMBINANT ANTIGENS IN INSOLUBLE FORM**

**FIELD OF THE INVENTION**

5 The present invention relates to an assay for the detection of various disease states which utilizes antigens in insoluble form and more particularly, for the detection of HIV infection. The assay of the present invention has a high sensitivity allowing for detection of antibodies to the HIV virus in the "window" period.

**BACKGROUND OF THE INVENTION**

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Enzyme immunoassays (EIA) and Western Blot assays (WB) have been routinely used for detecting HIV infection for several years. The implementation of these tests has significantly reduced the risk of transfusion-related HIV infection. However, some recent studies based on the detection of HIV DNA  
15 have demonstrated that some HIV infected individuals do not have any detectable amount of anti-HIV antibodies when the approved tests are performed. Data from several studies indicate that there is a "window period" estimated to span from a few weeks to several months or even several years between initial HIV infection and seroconversion. Cases of post-transfusion HIV infection have been  
20 reported from seronegative blood donors. This is assumed to be a consequence of donation during the "window" period on the part of these donors.

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To verify the presence of HIV-antibody in donors of reactive samples to EIA, the Western Blot assay has been used for confirmatory testings (Ulstrup, J.C. et al, Lancet i:1151-1152). In this assay, 6 to 9 characteristic bands indicating antibodies to HIV surface and core antigens are observed if antibodies to HIV-1 proteins are present (positive), and no bands if antibodies are absent (negative). However, a significant proportion of EIA repeatedly reactive samples react only to the HIV-1 gag-derived core proteins (p17, p24 and p55) on the WB test

(Kleinman, S., 1990, Arch. Pathol. Lab. Med. 114:298-303, Tribe D.E. et al, 1988, J. Clin. Microbiol. 26:641-647). This reactivity does not meet the definition of HIV-1 positive or negative in the criteria for WB tests and as a result these samples are labelled as HIV-1 WB indeterminates. Studies have  
5 established that 30 to 40 percent of EIA repeatedly positive donors are HIV-1 WB indeterminates, a figure that is typical in North America. Follow-up studies performed in regions of high prevalence of HIV show that while 95 to 99 percent of these are not infected with HIV, the remaining 1 to 5 percent of blood donors with HIV-1 WB indeterminate results are true seroconverters, usually at an early  
10 stage of infection (Busch, M.P. et al., 1991, Transfusion 31:129-137; Gallo D. et al, 1986, J. Clin. Microbiol. 23:1049-1051). In order to optimize the safety of transfusion, all donors with HIV-1 WB indeterminate results are deferred. The majority of donors with HIV-1 WB indeterminate results undergo needless anxiety, their deferral represents a significant loss of donors and recipients are  
15 still worried about contamination of blood taken from "window" period of seroconversion donors. A highly sensitive assay is thus also desirable in order to properly classify these indeterminate results.

Partially purified disrupted virus is used as an antigen for most currently  
20 licensed screening and confirmatory tests. Human cells are always used for culturing the HIV-1 virus (Dodd, R.Y. and C.T. Fang, 1990, Arch. Pathol. Lab. Med. 114:240-245). Recombinant proteins and synthetic peptides have been recently licensed for screening tests (Busch M.P. et al., 1991, Transfusion 31:129-137; Das P.C. et al., 1992, Trans Med 2:249-250; Ramirez E.P., 1992,  
25 J. Clin. Microbiol. 30:801-805). In theory, these antigens can provide more sensitive and definitive assays. However, most recombinant proteins are produced in E. coli and denatured during the purification and processing of the antigens. Also, a certain proportion of donors still show cross-reactivity to HIV core antigens (such as antigen p24), and sensitivity is limited to detecting very  
30 early HIV antibodies.

5 A serious drawback to the use of synthetic peptides is related to the fact that in some HIV-1 infected patients and seroconverters in high risk populations, the serum antibody titre is very low, or undetectable, either because of complex formation between p24 and antibodies or the loss of specific clones of antibody producing cells (Orskov, L.B., Eur. J. Clin. Microbiol. Inf. Dis. 8:614). Synthetic peptides, which in general only cover one or two epitopes, do not efficiently detect such low titre antibodies and furthermore have a limited ability to take on the natural three dimensional structures of antigen.

10 An immunofluorescence assay (IFA) has recently been licensed as an alternate confirmatory test for detecting HIV-1 antibodies (FDA Memorandum, 1992, Summer:56-67). IFA is rapid, simple and inexpensive. However, it is a subjective procedure requiring well trained personnel (Ascher, M.S. 1990, Arch. Pathol. Lab. Med. 114:246-248). This is a limitation for users and false positive  
15 and false negative results still occur in the IFA tests because of cross-reactivity of some antibodies to antigens on the human cells in which the HIV virus is cultured. Fixing of the infected cells before incubation also has been shown to lead to false positive and negative results (McHugh, T.M., 1986, Diagnos. Immunol. 4:233-240).

20 An assay based on immunofluorescence was recently developed to detect antibodies to HIV-1 by using flow cytometry (FIFA) (Sligh, J.M., 1989, Am. J. Clin. Path. 91:210-214). FIFA is a sensitive, quantitative test. In a typical FIFA protocol, HIV-1 infected cells are used directly for the test. However,  
25 false positive and false negative results may occur because of the HIV-1 antibody cross-reactivity to the antigens (such as HLA) on human cells in which the virus is cultured and used as antigens in FIFA. Another concern is with biohazards caused by the infectious virus which is a big limitation for users. Fixing the HIV-1 infected cells for inactivation of the virus before incubation has shown to  
30 lead to higher cross reactivity on the cells.

The present invention is directed to a flow cytometric immunofluorescence assay (r-FIFA) using insoluble forms of recombinant proteins expressed in an expression system such as the baculovirus system. In a preferred embodiment, r-FIFA is used for the detection of the HIV virus infection. Insoluble forms of recombinant HIV-1 proteins such as HIV-1 gag p45 protein, gag gp-41 chimeric proteins, HIV-1 precursor polyproteins pol 97 and gp160 are used as autologous carriers (in place of beads) and antigens to detect HIV-1 antibodies using flow cytometry. The baculovirus expression system has become a major recombinant protein production system because of several advantages over bacterial and mammalian systems including superior yield of recombinant protein, safety (baculovirus is not infectious to humans) and fidelity of its products.

In sensitivity comparison between r-FIFA and currently licensed tests, r-FIFA was found to be more sensitive, the average increase in sensitivity for early detection of HIV-1 infection being greater than 20 days. r-FIFA has permitted the detection and quantification of HIV-1 specific IgG, IgM and IgA antibodies during the window period. The use of HIV-1 recombinant proteins in an immunofluorescence assay (r-FIFA) solves the problems of antibody cross-reactivity to antigen on human cells and the biohazard concern with the original FIFA.

## SUMMARY OF THE INVENTION

In a broad embodiment, the invention relates to the use of an insoluble form of at least one recombinant protein as a carrier and antigen in a flow cytometric immunofluorescence assay for the detection of a disease state wherein said protein reacts with an antibody present in said disease state. In a preferred embodiment, the invention relates to the use of an insoluble form of at least one recombinant protein as a carrier and antigen in a flow cytometric immunofluorescence assay for the detection of HIV.

## BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood through the following detailed description of the preferred embodiment in conjunction with the accompanying drawings in which:

Figure 1 represents the construction of a recombinant baculovirus containing the HIV-1 gag gene sequence coding for gag-45 protein. The gag-45 coding region was isolated from the plasmid pHxB-2D (8). A ClaI-Bgl II fragment was modified and subcloned into pUC19 by ligation with two synthetic oligonucleotide linkers. The linker 1 contains a Bam HI site and the missing sequence including the translation initiation codon (ATG) at N-terminal of gag gene. The linker 2 creates a translation termination codon (TAA) followed by a Bam HI site. The Bam HI fragment was isolated from the pUC19-gag45 and inserted into the Bam HI site of transfer vector pAcYM1 (9). The recombinant transfer vector pAcYM1-gag 45 was used for co-transfection of SF9 cells with wild type AcNPV DNA and then the recombinant baculovirus gag45 gene was isolated to express the recombinant protein gag45;

Figure 2 is the nucleotide sequence of HIV-1 gag protein p45 (SEQ ID NO:1);

Figure 3 is the amino acid sequence of HIV-1 gag protein p-45 (SEQ ID NO:2);

Figure 4 is a schematic representation of the construction of recombinant HIV-1 gag gp41 proteins. Three gag-gp 41 chimeric proteins were constructed based on the truncated gag precursor p45 gene sequence. The gp41 coding region A (nucleotides 7737-8090), 118 a.a.), B (nucleotides 7923-8264, 114 a.a.) and C (nucleotides 7737-8264, 176 a.a.) were amplified by PCR with primers p<sub>1</sub>, p<sub>2</sub>, p<sub>3</sub> and p<sub>4</sub> which contain a Bgl II enzyme site at both ends and a stop codon TAA at the 3' end and then inserted respectively into the Bgl II enzyme site at 3' end

of gag-p45 coding sequence in the recombinant plasmid pAcYm1 gag p45. The chimeric DNAs were isolated and inserted into the Bam H1 cloning site of AcNPV transfer vector plasmid pVL 1393. The recombinant viruses gag-gp 41A, gag-gp 41B and gag-gp 41C were isolated and purified after co-transfection;

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Figure 5 is the nucleotide sequence (SEQ ID NO:3) and amino acid sequence of chimeric protein A (SEQ ID NO:4);

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Figure 6 is the nucleotide sequence (SEQ ID NO:5) and amino acid sequence of chimeric protein B (SEQ ID NO:6);

Figure 7 is the nucleotide sequence (SEQ ID NO:7) and amino acid sequence of chimeric protein C (SEQ ID NO:8); and

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Figure 8 represents the titration of HIV-1 seropositive plasma using gag-p45 as the antigen in r-FIFA. A: The flow cytometric histograms of the seropositive and seronegative plasma are presented showing HIV-1 specific antibody (IgG) positive signal (the line peaks) and the negative signal (solid grey peak). Each of the two line peaks represent the duplicates of the assay. The heavy overlapping of the duplicates indicates the high reproducibility of r-FIFA. B: The median fluorescence intensity of the HIV-1 positive sample (○) and the negative sample were plotted (●) over the dilutions (1:25 to 1:25 600). The median fluorescence intensity ratio of HIV-1 positive sample to HIV-1 negative sample (S/N) is highest at 1:25. C: The dilution of 1:25 was chosen for r-FIFA to detect IgG and IgM antibodies (B-IgG and B-IgM) to HIV-1 in a HIV-1 positive sample by double staining with goat anti-human IgG FITC and IgM R-PE;

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Figure 9 represents early HIV-1 antibodies detection in the samples of BB1 anti-HIV-1 seroconversion panel D by r-FIFA using two different antigens. The flow cytometric histograms show anti-HIV-1 antibody signals in those samples

(line peaks) and fluorescence signal (background) of the negative sample as control (solid grey peak);

5        Figure 10 represents detection of early HIV-1 antibodies (IgG and IgM) using r-FIFA in the samples from three individuals (PHL-A, PHL-B, PHL-C) who were infected but seronegative in the first bleed (upper row) by recently licensed screening tests. The numbers under IgG and IgM indicate the s/c value of the samples; 1.0 or greater is considered positive;

10        Figure 11 represents antibody response during the window period of HIV-1 infection. In this kinetic analysis of IgM and IgG antibody production the results are expressed by median fluorescence intensity ratio of sample to cut-off (s/c) value (left axis). The broken line represents the antibody s/c value level of 1.0. The s/c value on this line or higher is considered positive. The right axis  
15        represents the s/c (s/co) value of HIV-1 antigens. A typical primary immune response was found in panels J, K, D and R (A). The immune response pattern in panels E, H, P and Q (B) is different from the pattern seen in (a). IgG is the dominant antibody and remains at low level for a long period. (○) represents IgM antibody to HIV-1, (●) represents IgG antibody to HIV-1 and (▼)  
20        represents HIV-1 antigen;

Figure 12 represents the kinetics of the immune response during the window period of HIV-1 infection. The fluorescence histograms of samples in BB1 panel K represent IgG, IgM and IgA antibody production showing the phases of  
25        primary response at a very early stage of HIV-1 infection. IgA antibody in samples K1 and K2 are significantly stronger than the negative control, but they are still negative by our criteria because the s/c value is higher for IgA than for IgG;

30        Figure 13 represents antibody detection using antigen gag-gp41-C (p45-cc, a) and gag-p45 (p45,b) in r-FIFA. The sample tested had weak antibodies to HIV-1

core (45) and strong antibodies to gag-gp41 (cc) by Western Blot (IgG only) (a). The antigen gag-p45 (45,B) and antigen gag-gp41 (cc,B) were visualized by Coomassie blue staining of the polyacrylamide gel. The r-FIFA results (a,b) are shown to be concordant with Western Blot results;

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Figure 14 represents HIV-1 early antibody (IgG) detection by using the gag-p45 antigen and the antigen chimeric gag-gp41-C in r-FIFA. The results shown indicate that chimeric antigen gag-gp41-C detects the antibodies earlier and with a stronger signal than the antigen gag-p45 does during the early stage of HIV-1 infection;

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Figure 15 represents antibody response to HIV-1 RT precursor pol97 polyprotein tested by r-FIFA during early HIV-1 infection in the anti-HIV-1 seroconversion performance panel J(BB1). The axes represent the s/co value of HIV-1 antigens (right) and the sample to cut-off ratio (s/c) of antibody to pol97 (left). The broken line represents the antibody s/c value of 1.0; and

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Figure 16 represents the detection of antibodies to HIV-1 gag-p45 particles by indirect immunofluorescence. The HIV-1 antibody bound gag-p45 particles were stained with goat antihuman IgG FITC and IgM RPE conjugates showing the HIV-1 specific antibodies IgG (green fluorescence) and IgM (orange yellow fluorescence).

20

## DESCRIPTION OF THE PREFERRED EMBODIMENT

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The present invention relates to the use of an insoluble recombinant protein in a flow cytometric immunofluorescence assay, hereinafter referred to as r-FIFA, for the detection of antibodies. In the examples that follow, the use of insoluble forms of the HIV-1 gag precursor p55, termed p45 protein, which includes p17, p24 and part of p16 and chimeric particles of gag-gp41 fusion proteins; the gp160 and pol97 proteins for the early detection of HIV as well as

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the hepatitis B core antigen for the detection of hepatitis B is described.

Recombinant proteins for the present invention are prepared in the baculovirus expression system since the baculovirus expression system has several advantages over bacterial and mammalian systems. These include a superior yield of recombinant protein, safety and fidelity of the products. Glycosylation, myristoylation, proteolytic processing and other post-translational modifications that occur in baculovirus expressed proteins are similar or identical to the native HIV-1 proteins and to HIV-1 proteins derived from mammalian cell culture systems.

When expressed in insect cells, baculovirus encoded rp45 exhibits two molecular forms, an insoluble particle form and a soluble protein. The particle form of gag protein consists of a membrane-enveloped corelike particle released into the medium by budding at the plasma membrane. Both of the two forms of gag rp45 are myristoylated (Mervis, R.J., 1988, J. Virol. 62:3993-4002). HIV-1 gag particle is 100 to 120 nm in diameter. It is a good carrier for inserting immunoreactive domains of other proteins, such as neutralizing epitopes of gp120 by using recombinant DNA techniques, thereby extending the immunoreactivity spectrum of this gag protein.

Standard performance panels obtained from BBI (Boston Biomedica Inc.) were used for the evaluation of r-FIFA. These performance panels are made available to enable manufacturers and users to test and assess their anti-HIV-1 test systems, especially with regard to specificity, reproducibility and sensitivity. BBI provides comprehensive data for comparative analysis. Each set of BBI panels includes 6 to 14 aliquots assembled from a repository of frozen sera or plasma units. An anti-HIV-1 low titer performance panel (PRB-104), anti-HIV-1 seroconversion panels D(PRB 904), E(PRB 905), H(PRB-908), J(PRB 910), K(PRB-911), P(PRB-916), Q(PRB-917), R(PRB-918) and three HIV-1 seroconversion specimens provided by PHL were tested and analyzed by r-FIFA. To confirm

the specificity of r-FIFA, 295 plasma or serum samples from random blood donors, 105 Western Blot positive samples and 138 E1A reactive, Western Blot indeterminate samples from The Canadian Red Cross Society, National Testing Laboratory were also tested.

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### EXAMPLE 1

#### Construction of recombinant baculoviruses for the expression of HIV-1 gag p45 and chimeric gag-gp41 proteins

10        Recombinant baculoviruses were constructed to express HIV-1 gag p45 and chimeric gag-gp41 proteins gag-gp41 A, gag-gp41 B and gag-gp41 C. The gag p45 coding region (including intact p17, p24 and part of p16 coding sequences) was isolated from the plasmid pHxB-2D (Ratner *et al.*, 1985, Nature 313, 277-284). A ClaI-Bgl II fragment was modified and subcloned into pUC19 by using  
15        synthetic oligonucleotide linkers. Linker 1 contains a Bam HI site and the missing sequence including the translation initiation codon (ATG) at the N-terminal of the gag gene. Linker 2 created a translation termination codon (TAA) followed by a Bam HI site. The Bam HI fragment was isolated from pUC19-gag p45 and inserted into the Bam HI site of transfer vector pAcYM1 as  
20        illustrated in Figure 1. The recombinant plasmid pAcYM1-gag p45 was used for co-transfection of SF9 cells with wild type AcNPV DNA and then the recombinant baculovirus gag-p45 was isolated to express the recombinant protein gag p45. Three gag-gp41 chimeric proteins were constructed and are referred to as gag-gp41 A, gag-gp41 B and gag-gp41 C and represented in schematic form  
25        in Figure 4. The gag coding sequence in the three constructions is the same as gag p45. The gp41 coding sequences A (nucleotides 7737-8090, 118 a.a.), B (nucleotides 7923-8264, 114 a.a.), and C (nucleotides 7737-8264 176 a.a.) were inserted respectively at the Bgl II site at the end of the gag-p45 gene. The nucleotide and amino acid sequences of gag-p45 (SEQ ID:1 A and SEQ ID:2  
30        respectively) are provided in Figures 2 and 3. The nucleotide and amino acid sequences of the three chimeric proteins are provided in Figures 5, 6 and 7 (SEQ

ID:3 and 4 for Protein A, SEQ ID:5 and 6 for protein B and SEQ ID:7 and 8 for protein C). The chimeric DNAs were isolated and inserted into the Bam H1 cloning site of transfer vector plasmid pVL 1393 and cotransfection was performed using the BaculoGold system (Pharminogen). Recombinant viruses were isolated as chimeric gag-gp41 A, gag-gp41 B and gag-gp41 C.

### Construction of Recombinant HIV-1 Gag/Env Chimeric Proteins

Three gag/env chimeric proteins were constructed and were referred to as chimeric A, B, and C as shown in Figure 4. The gag DNA sequence in the three proteins was the same. That sequence contained a 5' truncated HIV-1 gag protein (p45) sequence excluding the TAA termination codon. In addition, chimeric A contained 118 a.a. env sequences (nucleotides 7737-8090 SEQ ID:3). Chimeric B contained 114 a.a. env sequences (nucleotides 7923-8264 SEQ ID:5) and chimeric C contained 176 a.a. env sequences (nucleotides 7737-8264 SEQ ID:7). The chimeric DNA was inserted into the Bam H1 restriction site of plasmid pVL1393 and cotransfection was performed using the BaculoGold transfection system. The nucleotide sequences of the three chimeric proteins are provided in Figures 5, 6 and 7.

#### a) Amplification of HIV-1 env regions

About 100ng of HIV-1 plasmid DNA (pHxB-20) was used in the polymerase chain reaction (PCR). Amplification was performed using primers p1 and p2 to amplify the env region of chimeric A. Primers p3 and p4 were used to amplify the env region of chimeric B and primers p1 and p4 were used to amplify the env region of chimeric C as shown in Figure 4. PCR was performed using the Perkin Elmer Cetus amplification kit and their cyclor (Gene Amp PCR System 9600). The amplification reaction was denatured by heating to 95°C for 20 seconds, then annealed at 68°C for 15 seconds and extended at 72° for 45 seconds. A total of 30 cycles were performed.

**b) Cloning and Bgl II digestion**

The PCR product of each of the env sequences was inserted into the TA cloning vector using the manufacturer's procedures (TA cloning kit, Invitrogen).  
5 Using the same kit, transformation of E. coli competent cells was performed and recombinant white colonies were picked up and grown in LB medium. DNA was extracted from the cells using published procedures and was used for digestion. This DNA was digested to completion with Bgl II (Gibco BRL), which incises twice in the plasmid at each of the two primers and hence releases the PCR  
10 product and introduces Bgl II sticky ends. This DNA was then ligated to the Bgl II cut pAcyM1 (at position 1310 nucleotides) vector, which contains the truncated HIV-1 gag DNA sequence. The ligated DNA was then introduced into E. coli competent cells and recombinant white colonies were grown in LB media. This DNA was extracted and used for Bam H1 digestion.

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**c) Bam H1 digestion and cloning into pVL1393**

The plasmid pAcYM 1, which contained the recombinant gag/env sequences, was digested with Bam H1 (Gibco BRL) in order to release the entire insert.  
20 This DNA was then ligated to Bam H1 cut pVL1393 (BaculoGold transfection vector). After ligation and transformation of E. coli competent cells, white colonies were grown in LB and DNA was extracted and purified.

25

**d) Cotransfection**

An equivalent of 5 $\mu$ g of recombinant pVL1393 was used in the cotransfection procedure. This procedure was performed as recommended by the manufacturer (BaculoGold transfection kit, Pharmingen).

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It will be understood by a person skilled in the art that while the present application only describes the preparation of three chimeric proteins, other

chimeric proteins could be made and used as well in the immunofluorescence assay. Chimeric proteins containing conservative regions and having at least an antigenic or immunoreactive domain or epitope could also be used for the present invention.

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## EXAMPLE 2

### Construction of Recombinant Baculovirus to Express HIV-1 gp160

A 5' end primer and 3' end primer having the following sequences:

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5' end primer

CGC TGA TCA ATG AGA GTG HAG GAG AAA TAT CAG C

3' end primer

CGC TGA TCA TTA TAG CAA AAT CCT TTC CAA GCC C

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were designed to amplify the entire HIV-1 gp160 coding sequence (env open reading frame) (Ratner *et al.*, Nature 313, 277-284) by polymerase chain reaction (PCR). In addition, a BcL1 enzyme recognition sequence was included in each of the primers to facilitate cloning of the coding sequence into a vector plasmid. After the gp160 gene was cloned and inserted into a baculovirus transfer vector pvl 1393, by techniques well known in the art, the co-transfection was performed using the procedure provided by the Baculo-Gold System (Pharmigen). The recombinant virus was isolated to express a fully glycosylated gp160 polyprotein, the precursor of HIV-1 env gene products.

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## EXAMPLE 3

### 25 Construction of Recombinant Baculovirus to Express HIV-1 pol 97

The recombinant virus expressing pol 97 was constructed as disclosed in Hu *et al.*, 1991, Proc. Natl. Acad. Sci., 88, 4596-4600.

**EXAMPLE 4****SF9 Cell Culture and HIV Recombinant Protein Production**

5 Spodoptera frugiperda (Sf9) cells are grown in monolayer (175 cm<sup>2</sup> Falcon tissue culture flasks) or roller bottle (850 cm<sup>2</sup> cell culture bottles) cultures. The medium used for either culture condition is Gibco BRL Sf900 medium supplemented with 100 U/ml each of sodium penicillin G and streptomycin sulphate (Gibco BRL) or Sigma TNM-FH medium supplemented with 10% (v/v) fetal bovine serum (Gibco BRL) and 100 U/ml each of the above antibiotics.

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The cells are infected at an moi of 5 - 10 with recombinant baculovirus (Autographa californica). Harvest of the intracellular protein (protein contained within the cells prior to cell lysis) was done using the same procedure for both the monolayer and roller bottle cultures after an optimum time of 72 hours post infection. The cells were released from the surface of the flask or bottle and the suspension was spun down for 10 minutes at 250 x g at ambient temperature on a Beckman GP centrifuge. The cell pellet and the supernatant were then further processed. The supernatant (extracellular protein) was transferred to Beckman Ultraclear tubes and spun down on a Beckman L8-80M ultracentrifuge at 26,000 rpm (SW28 rotor) at 20°C for 1.5 hours. The pellet was resuspended in 1 - 2mL of phosphate buffered saline (PBS, 2.67 mM KCl, 1.15 mM KH<sub>2</sub>PO<sub>4</sub>, 137.9 mM NaCl, 8.06 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.4) and then stored at -70°C until use.

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The cell pellet was washed in 30mL of PBS and spun down again in the GP centrifuge. The pellet was resuspended in 10mL of PBS then sonicated on ice for 45 - 50 seconds at 40% power (Cole-Parmer Ultrasonic Homogenizer 4710). The cell lysates were spun down on the GP centrifuge at 900 x g for 10 minutes. The pellet fraction I, was resuspended in 1 - 2 mL of PBS and was held at 4°C until use. The supernatant was spun down in a Sorvall RC-5 centrifuge with an SS-34 rotor at 10,000 rpm at 4°C for 30 minutes. The pellet fraction II, was resuspended in 1 - 2 mL of PBS and held at 4°C until use. The supernatant was

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spun down on the Beckmann ultracentrifuge under the same conditions as the extracellular protein. The resulting pellet, fraction III, was resuspended in 1 - 2mL of PBS and the supernatant was discarded. Fractions II and III were combined to form the intracellular protein stock. The resulting pellet was further purified by sucrose gradient (20% - 60%) ultracentrifugation at 26000K rpm (SW28 rotor) for 3 hours at 20°C. The purified insoluble proteins were washed in PBS and then spun down on an IEC Micro-MB centrifuge for 5 minutes at room temperature. The pellet was washed twice more then stored at -70°C until further use.

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## EXAMPLE 5

### Flow cytometry - r-FIFA assay for the detection of HIV-1 antibodies

#### 1. Flow cytometry

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Flow cytometric analysis was performed on a Becton Dickinson FACSort equipped with an argon ion laser tuned at 488 nm. Data acquisition was done with Lysus II 2.0 software, version 1.1 (Becton Dickinson). Forward light scattering, orthogonal light scattering and two fluorescent signals were determined on logarithmic settings for each of 20,000 events and stored in data files. Detector settings had been determined and stored in data files for recall by the operator. Data analysis was also performed with the Lysis 2.0 software. A two dimensional dot intensity plot of forward light scatter versus orthogonal light scatter was observed on ungated events. A region (R1) was set on the dot plot and single parameter histograms of FL1 (green emission for FITC is 530nm) and FL2 (red emission for R-PE is 585nm) were examined. The median fluorescence channel was used to determine positivity of the test samples. This analysis procedure has also been automated and stored as a command file.

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#### 2. r-FIFA procedure

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In 1.5mL microcentrifuge tubes (Sarstedt) 4μL of control or sample (plasma or serum) and a pre-determined amount of recombinant protein were combined

along with PBS (containing  $\text{NaN}_3$ ) to a final volume of  $100\mu\text{L}$ . Most of the tests were done with a mixture of gag-p45 and gag-gp41-B recombinant proteins. The rest were done with a single antigen as indicated. The tubes were gently vortexed then incubated at ambient temperature for 20 minutes on a rocker. The mixture was washed in  $1\text{mL}$  of PBS/tube and spun down for 5 minutes, ambient temperature at  $12\,700 \times g$  on an IEC Micro-MB centrifuge (fixed speed). The supernatant was aspirated and the pellet was gently resuspended in  $1\text{mL}$  PBS and spun down as before. The supernatant was aspirated and  $10\mu\text{L}$  of FITC and/or R-PE labelled antibody is added. The mixture was incubated for 20 minutes at ambient temperature in the dark then washed two times with  $1\text{ ml/tube}$  of PBS as before. After the second aspiration,  $500\mu\text{L}$  of PBS was added to each sample tube and the mixture was sonicated for 10 seconds at the 40% power setting with a  $2\text{mm}$  diameter probe attachment. The contents were then transferred to  $12 \times 75\text{ mm}$  polystyrene tubes (Becton-Dickinson, specific for the flow cytometer) and stored for 2 hours in the dark at ambient temperature then read on the flow cytometer.

To determine the cut-off values of fluorescence intensity for r-FIFA for each of the antigens (gag-p45, gag-gp41-C, pol97, and the mixture of gag-p45 and gag-gp41-B proteins) 100 normal donors were tested by using double staining with IgG FITC and IgM R-PE. Each sample was tested in duplicate and reported in relation to the mean value of a known seronegative control (S/N). The cut-off value was calculated by taking the mean S/N value of the population ( $\bar{x}$ ) and adding two standard deviations (2 SD) as described by Sligh (Sligh, 1989, Amer. J. Clin. Pathol. 91, 210-214). For example, the cut-off value of fluorescence intensity of gag-p45 was calculated as follows:

|          |                                                              |
|----------|--------------------------------------------------------------|
| IgG FITC | $\bar{x} \text{ S/N} = 0.960$                                |
|          | $1 \text{ SD} = 0.266$                                       |
|          | $\text{cut-off} = \bar{x} \text{ S/N} + 2 \text{ SD} = 1.48$ |

IgM R-PE  $\bar{x}$  S/N = 1.666

1 SD = 0.581

cut-off =  $\bar{x}$  S/N + 2 SD = 2.83

5 IgA R-PE  $\bar{x}$  S/N = 1.270

1 SD = 0.604

cut-off =  $\bar{x}$  S/N + 2 SD = 2.48

10 Sample to cut-off (s/c) ratios of 1.0 or greater are considered positive.

### EXAMPLE 6

#### Sensitivity and Reproducibility of r-FIFA

15 In order to determine the optimal serum or plasma dilution required for the sensitivity test of r-FIFA, an HIV-1 seropositive plasma and a control (negative plasma) were treated with gag-p45 as the antigen. Fig 8 shows the fluorescence histograms of the samples at different dilutions. The fluorescence intensity of the positive sample sharply decreased as the dilution increased. It was positive at  
20 1:6400 (s/c = 1.25). The 1:25 dilution was shown to be the optimal sample dilution for r-FIFA and this dilution was used for further experiments.

Table 1 summarizes the results of r-FIFA for detection and analysis of eight BBI anti-HIV-1 seroconversion panels and compares the sensitivity of r-FIFA  
25 with currently licensed tests. Anti-HIV-1 antibodies were detected by r-FIFA in the first bleed of seven BBI panels. The average increase in sensitivity was greater than 20 days. Fig. 9 illustrates the fluorescence histograms of samples in Panel D. The HIV-1 antibody (IgM) had become positive three months before antibodies were detected by EIA. The antibody titer dropped in sample D3  
30 possibly because of HIV antigen-antibody complex formation.

To evaluate the reproducibility of r-FIFA, two antigens gag-p45 and gag-gp41-C were used for the analysis of panel D. The results of the two assays were almost identical. The duplicates of each assay were highly reproducible as shown by the overlapping of two line peaks which represent the fluorescent intensity of each sample. In addition to the BBI panels, three HIV-1 seroconversion panels from PHL were evaluated in a blind test to confirm the sensitivity of r-FIFA (Fig.10). The first bleed of all three patients was seronegative in EIA and WB tests. However, IgG and IgM HIV-1 antibodies were detected by r-FIFA. The antibody response pattern of the three panels suggests that the patients were infected recently since IgM was the dominant antibody. The first bleed from the patient PHL-A (who donated blood for a transfusion) was negative by EIA in 1988. The recipient was infected by the seronegative but HIV-1 infected blood. The donor was identified as HIV-1 positive by testing in 1993 with a recently licensed test which used conjugated second antibody to both human IgG and IgM. The 1988 sample was still negative by this test but the 1993 sample was strongly positive. In r-FIFA, both the 1988 and the 1993 samples were positive for IgG and IgM.

Furthermore, to compare the sensitivity of r-FIFA with that of FDA licensed confirmatory tests including WB, and radioimmunoprecipitation (RIPA), the BBI anti-HIV-1 low titer panel was tested with r-FIFA using each of the antigens gag-p45, gag-gp41 chimeric proteins and po197. The results are summarized in Table 2 and show that r-FIFA is more sensitive than any of the FDA licensed confirmatory tests. The antibodies to gag and env or to all three of these antigens were positive by r-FIFA in 12 of the 14 samples. Only 8 to 10 were positive in the four confirmatory tests. The results show that the po197 is an excellent antigen for HIV-1 antibody detection. Antibodies to po197 were detectable in 8 out of the 14 samples in r-FIFA. However, the antibodies to pol gene products (p68, p51 and p31) were poorly detected in the FDA licensed confirmatory tests. Only 1 to 4 of the 14 samples were found to have antibodies to pol proteins on WB and RIPA. It has been reported that antibodies to gag-

encoded proteins appear first, followed closely by those antibodies to env glycoproteins, then pol reactivity to p66 appear on WB. Our studies show that the antibodies to po197 were detected as early as those to gag-p45 and gag-gp41 chimeric proteins as shown in Fig. 15. This provides evidence to the effect that antibodies to pol gene products are one of the important serological markers for early diagnosis of HIV-1 infection and monitoring of HIV-1 infection disease progression.

#### EXAMPLE 7

##### Specificity of r-FIFA

295 random donor samples, 105 EIA/WB positive samples and 128 EIA repeat reactive or positive/WB indeterminate samples were tested by r-FIFA. Only 1 of the 295 random donor samples was positive. One of the positive samples from the random donor was negative in EIA but was positive by radioimmunoprecipitation (r-RIPA). All 105 positive samples were positive by r-FIFA. Only 30 of 128 (23%) EIA repeat reactive WB indeterminate samples were still positive. These results indicate that r-FIFA's specificity is higher than that of EIA and WB.

#### EXAMPLE 8

##### Antibody response during the window period

Fig. 11 demonstrates the patterns of HIV-1 antibody production in eight BBI panels. A typical primary response was found in panels J and K. Panels D and R show a similar pattern of antibody production. However, in panels E, H, P and Q, the HIV-1 IgG antibody remained at a certain level for a long period (over two or three months) prior to seroconversion as detected by licensed tests. The IgM antibody became positive later at a low level or at the same time (panel Q) as did IgG antibodies. The pattern of E, H, P and Q is apparently different from the pattern of D, J, K and R. Presumably these individuals were not recently infected by HIV-1. For some reason, virus-antigen concentration rose,

stimulating the antibody response. The anti-HIV-1 IgG antibodies jumped up to a high level in a shorter time (less than a week as seen in Q and R) indicating a possible secondary immune response. Fig. 12 shows the fluorescence histograms of the samples in panel K, indicating the kinetics of the specific IgG, IgM and IgA production during the window period. The IgM antibodies were detected first before the antigen was detected, and then IgA and IgG antibodies appeared about two weeks after the antigen level peaked. The IgM antibody response to HIV-1 proteins overlapped with the HIV-1 antigen peak and started before the antigen was detected.

#### EXAMPLE 9

##### Differences in antibody response to different antigens gag-p45, gag-gp41-C and pol197 in r-FIFA

Chimeric gag-gp41-C binds more IgG antibodies than gag-p45 and detects them earlier. Little difference was found in detection of HIV-1 IgM antibodies (Fig. 13 and 14). HIV-1 IgG antibodies to pol197 were detected as early as gag-gp41-C (Fig.15). IgM antibody to pol197 was very low during this "window" period.

#### EXAMPLE 10

##### Comparison of r-FIFA Assay with gag-p45, gag-gp 41-C, pol197, gp 160 and Mixture of gag-45, pol197 and gp 160

In a preferred embodiment, a mixture of equal amounts of gag-p45, pol197 and gp 160 is used in the r-FIFA. The proteins were prepared as described in the previous examples and the r-FIFA was conducted as previously described. An anti-HIV-1 low titer performance panel (PRB 104) was utilized. The results are reported in Table 3. The testing with a mixture of all three proteins was carried out with 5 samples. The results with the mixture were positive in all instances. This shows the sensitivity of the procedure with a mixture of the proteins. In instances where the results were negative for each of the four proteins individually, the results with the mixture were negative as well (sample 10) and where the results were positive with each of the proteins individually, the results of the mixture were positive as well (sample 7). The use of a mixture of the

proteins is advantageous in a situation where the results with each of the proteins all not all positive or negative (samples 3, 11 and 14). In all instances where some of the individual results were negative, the use of a mixture of proteins allowed a final determination and gave positive results. The gag, gp and pol polyproteins cover over 90% of the viral structural proteins and allow r-FIFA to be used as a confirmatory test because of its high sensitivity, specificity and its ability to identify antibodies directed to individual HIV-1 polyproteins.

#### EXAMPLE 11

##### Flow Cytometry - r-FIFA Assay for the Detection of Hepatitis B Antibodies

The Hepatitis B core antigen was prepared as is known in the art and as is described for example in Takehara *et al.*, 1988, J. Gen. Virology, 69:2763-2777. Recombinant baculoviruses were constructed to express the hepatitis B core antigen in the same manner as described in Example 1 in relation to HIV-1 gag p45 and chimeric gag-gp 41 proteins. The r-FIFA assay for the detection of antibodies to Hepatitis B core antigens was conducted as set out in Example 5 in relation to the detection of HIV-1 antibodies. The results of a comparison of sensitivity between licensed EIA and RIA tests for Hepatitis B and r-FIFA using standard BBI panels are set out in Table 4.

The above examples demonstrate that r-FIFA is more sensitive than licensed screening and confirmatory tests for detection of early antibodies to HIV-1. At least one class of anti-HIV-1 specific IgG, IgM or IgA was detected during the window period of HIV-1 infected individuals. We have created a unique way to detect early antibodies using insoluble forms of recombinant proteins as autologous carriers through a flow cytometer. Two patterns of antibody response were observed, which may represent two types of immune response: primary and secondary.

On average for the tests which were in use in 1990 the window period was 45

days. Since then the tests have increased sensitivity for HIV antibody detection resulting in earlier detection of seroconversion and a significant decrease of approximately 12-13 days in the length of the infectious window period (Dodd, 1990, Arch. Pathol. Lab. Med. 114, 240-245). An analysis of the records made in 19 American National Red Cross regions in 1992 and 1993 showed that the window period estimated using third generation tests was 25 days on average Petersen *et al.*, 1994, Transfusion 34,283-289). As shown in Table 1, the average increase in sensitivity using the method of the present invention was greater than 20 days. The r-FIFA of the present invention is a novel serological test, which uses insoluble polyproteins. The insoluble antigens are easily processed and purified without denaturation, keeping intact their natural molecular folding which may optimize the presentation of antigen epitopes. This may be one of the explanations to account for r-FIFA's higher sensitivity in detection of HIV-1 early antibodies. Furthermore, an agglutination effect involving the reaction between IgM antibodies and the antigens in r-FIFA may enhance the sensitivity for detection of specific antibodies because all the insoluble polyproteins used are particulate antigens. This is demonstrated in Figure 16. In general, IgM antibodies are more efficient in agglutination than IgG antibodies because of their size as well as their additional binding sites.

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The assay of the present invention (r-FIFA) is much more sensitive than any of the currently licensed tests used for analysis of BBI seroconversion panels to detect very early HIV-1 antibodies during the "window" period. Both anti-HIV-1 specific IgG and IgM have been found prior to the seroconversion of seroconversion panels. Some of them contain primarily anti-HIV-1 IgG or IgM. Some individuals have both anti-HIV-1 IgG and IgM. This appears to depend on the time of primary infection, appearance of antigenemia and the situation of the immune system of the HIV-1 infected individuals. Detection, quantification and differentiation of anti-HIV-1 antibodies in the "window" period have permitted a further understanding of the immune response and HIV-1 immunopathogenesis in this "immunological silent" period. The development of

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r-FIFA has allowed the use of insoluble proteins as antigens to detect antibodies. These insoluble proteins can be easily produced on a large scale using a baculovirus expression system.

5           While the r-FIFA procedure for use in the detection of HIV-1 infection is described in relation to gag p45, gag-gp 41, gp 160 and pol97 recombinant proteins, it will be understood that a mixture of these proteins as exemplified in Example 10 can be used as well as any number of other proteins and need not be recombinant proteins prepared in the baculovirus expression system. The  
10           proteins are not limited to those specifically exemplified. While the examples and the results set out above pertain to the use of the recombinant proteins in the detection and treatment of HIV-1, it is understood that the examples are not meant to be limiting. The invention would have applicability to the detection and treatment of HIV-2 using an insoluble form of a protein equivalent to gag p45 in  
15           HIV-1 as well as have applicability in the detection and treatment of other viral infections. This is illustrated in Example 11 where r-FIFA is used in the detection of hepatitis B. The invention also has applicability in the detection of other viral infections such as hepatitis C, HTLV-1/2 (human T-cell leukemia) or HTLV-II and has applicability for any virus-mediated condition where the virus  
20           proteins exist in insoluble form. Indeed, it is not limited to the detection of viral infections but has application for the detection of any disease state where an insoluble form of an antigen can be used in the assay of the present invention for the detection of antibodies. It is also contemplated that chimeric proteins can be constructed comprising several antigenic domains so that one test could be used  
25           for the detection of several disease states, for example, HIV-1/2, HTLV-1/2, hepatitis B and hepatitis C.

          Furthermore, it is also understood that where recombinant proteins are used in the assay of the present invention, any expression system sharing the  
30           advantages of the baculovirus expression system could also be used.

All publications referred to in the disclosure are hereby incorporated by reference.

5 While the present invention has been described in connection with a specific embodiment thereof and in a specific use, various modifications will occur to those skilled in the art without departing from the spirit and scope of the invention as set forth in the appended claims. I therefore wish to embody within the scope of the patent which may be granted hereon all such embodiments as reasonably and properly come within the scope of my contribution to the art.

Comparison of the Sensitivity Between r-FIFA and Licensed Tests for HIV-1 Antibodies in Anti-HIV-1 Seroconversion Panels (BBI)

| Code | # of Members | Time Span (Days) | Members Antigen + | Antibody Reactivity of Each Panel Member |     |     |     |     |     |     |   |   |    | Tests         |
|------|--------------|------------------|-------------------|------------------------------------------|-----|-----|-----|-----|-----|-----|---|---|----|---------------|
|      |              |                  |                   | 1                                        | 2   | 3   | 4   | 5   | 6   | 7   | 8 | 9 | 10 |               |
| D    | 5            | 101              | 0                 | -                                        | -   | -   | +   | +   | +   |     |   |   |    | EIA<br>r-FIFA |
| E    | 10           | 126              | 2                 | +                                        | +   | (+) | +   | +   |     |     |   |   | +  | EIA<br>r-FIFA |
| H    | 6            | 28               | 0                 | -                                        | -   | -   | (+) | -   | +   | (+) | + | + | +  | EIA<br>r-FIFA |
| J    | 7            | 40               | 0                 | +                                        | +   | +   | +   | (+) | +   |     |   |   |    | EIA<br>r-FIFA |
| K    | 10           | 36               | 0                 | -                                        | -   | -   | (+) | (+) | (+) | (+) | + | + | +  | EIA<br>r-FIFA |
| P    | 6            | 35               | 1                 | -                                        | -   | -   | -   | -   | +   | +   |   |   |    | EIA<br>r-FIFA |
| Q    | 7            | 72               | 5                 | +                                        | +   | +   | -   | -   | (+) | +   | + |   |    | EIA<br>r-FIFA |
| R    | 6            | 21               | 2                 | -                                        | (+) | (+) | +   | +   | +   | +   |   |   |    | EIA<br>r-FIFA |

EIA data shown in this table is a summary of 13 licensed test results from BBI. Both anti-HIV-specific IgG and IgM were detected by r-FIFA using fluorescence conjugated second antibodies to human IgG and IgM. The anti-HIV-1 antibodies IgG and IgM were detected separately by double staining with IgG FITC and IgM R-PE. If the s/c value  $\geq 0.9$  but  $< 1.0$  it is considered to be weak reactive (+); the s/c value of 1.0 or greater is considered positive +. The weak reactivity (+) by EIA means that the sample is positive in some tests and negative in others.

TABLE 1

Comparison between r-FIFA and FDA licensed confirmatory tests based on sensitivity for detection of antibodies to HIV-1 proteins encoded in the three open reading frames of HIV-1 genome in the anti-HIV-1 low titer panel PRB104.

| PRB104 LD.<br>Number | FDA Licensed Tests |  |     |  |     |  |                    |  |     |  |     |  |                    |  |      |  |     |  |                 |  |     |  |      |  | HIV-1 r-FIFA |  |     |  |     |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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|                      | BioRad WB*         |  |     |  |     |  | Ortho/Cambridge WB |  |     |  |     |  | Organon Teknika WB |  |      |  |     |  | HIV-1 RIPA RL15 |  |     |  |      |  |              |  |     |  |     |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|                      | gag                |  | pol |  | env |  | Res.               |  | gag |  | pol |  | env                |  | Res. |  | gag |  | pol             |  | env |  | Res. |  | gag          |  | pol |  | env |  | Res. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|                      |                    |  |     |  |     |  |                    |  |     |  |     |  |                    |  |      |  |     |  |                 |  |     |  |      |  |              |  |     |  |     |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

- 27 -

Detection of Antibodies to HIV-1 In Samples of Anti-HIV-1 Low Titer Performance Pabels (PRB104) using Different Antigens

| PRB104 I.D.<br>NUMBER | ANTIGENS    |                |           |           |                                            |
|-----------------------|-------------|----------------|-----------|-----------|--------------------------------------------|
|                       | gag-p45 s/c | gag-gp41-C s/c | pol97 s/c | gp160 s/c | mixture of gag-p45,<br>pol97 and gp160 s/c |
| 01                    | 1.64        | 1.18           | 1.10      | 1.17      | ND                                         |
| 02                    | 1.35        | 1.20           | 0.80      | 1.24      | ND                                         |
| 03                    | 1.11        | 1.29           | 0.87      | 1.00      | 2.30                                       |
| 04                    | 1.31        | 1.31           | 1.10      | 1.37      | ND                                         |
| 05                    | 1.49        | 1.08           | 1.47      | 1.03      | ND                                         |
| 06                    | 2.20        | 1.22           | 1.70      | 1.23      | ND                                         |
| 07                    | 1.27        | 1.00           | 1.80      | 1.00      | 1.36                                       |
| 08                    | 1.36        | 1.06           | 1.24      | 1.22      | ND                                         |
| 09                    | 1.34        | 1.00           | 1.32      | 1.00      | ND                                         |
| 10                    | 0.89        | 0.63           | 0.61      | 0.72      | 0.71                                       |
| 11                    | 1.45        | 1.04           | 0.80      | 1.00      | 1.46                                       |
| 12                    | 1.38        | 1.06           | 0.79      | 1.16      | ND                                         |
| 13                    | 1.59        | 0.93           | 1.47      | 1.22      | ND                                         |
| 14                    | 1.09        | 0.78           | 0.58      | 0.78      | 1.16                                       |
| 15                    | 0.81        | 0.77           | 0.86      | 0.54      | ND                                         |

TABLE 3

**Comparison of Sensitivity Between Licensed Tests and r-FIFA in  
Detection of Antibodies to HBc**

| MEMBER<br>I.D.<br>NUMBER | MATRIX | ANTI-HBc<br>IgM ABBOTT<br>EIA PROC A<br>(S/CO)<br>BBI |     | ANTI-HBc<br>IgM ABBOTT<br>EIA PROC B<br>(S/CO)<br>BBI RL1 |      | ANTI-HBc<br>IgM ABBOTT<br>RIA PROC A<br>(S/CO)<br>BBI RL1 |      | ANTI-HBc<br>IgM SORIN<br>EIA<br>BBI |     | HBsAg<br>ABBOTT RIA<br>PROC A<br>(S/CO)<br>BBI |      | ANTI-HBs<br>ABBOTT EIA<br>PROC B<br>(S/CO)<br>BBI |      | ANTI-HBc r-FIFA<br>IgG (s/c) IgM (s/c) |  |
|--------------------------|--------|-------------------------------------------------------|-----|-----------------------------------------------------------|------|-----------------------------------------------------------|------|-------------------------------------|-----|------------------------------------------------|------|---------------------------------------------------|------|----------------------------------------|--|
|                          |        |                                                       |     |                                                           |      |                                                           |      |                                     |     |                                                |      |                                                   |      |                                        |  |
| PHE201-01                | P      | 4.5                                                   | 4.8 | 4.7                                                       | 5.8  | 5.3                                                       | 9.2  | 8.2                                 | 114 | 0.2                                            | 5.6  | 2.7                                               | 5.6  | 2.7                                    |  |
| PHE201-02                | P      | 4.0                                                   | 3.9 | 4.6                                                       | 4.8  | 5.1                                                       | 6.0  | 9.9                                 | 99  | 0.2                                            | 8.7  | 2.71                                              | 8.7  | 2.71                                   |  |
| PHE201-03                | P      | 13.0                                                  | 5.5 | 4.6                                                       | 11.5 | 12.7                                                      | 13.1 | 12.0                                | 100 | 0.2                                            | 7.33 | 6.09                                              | 7.33 | 6.09                                   |  |
| PHE201-04                | P      | 0.4                                                   | 0.5 | 0.6                                                       | 0.5  | 0.5                                                       | 0.6  | 2.2                                 | 131 | 0.4                                            | 0.58 | 0.52                                              | 0.58 | 0.52                                   |  |
| PHE201-05                | P      | 6.5                                                   | 5.1 | 4.6                                                       | 5.0  | 6.0                                                       | 8.2  | 10.1                                | 5   | 0.2                                            | 6.93 | 4.66                                              | 6.93 | 4.66                                   |  |
| PHE201-06                | P      | 3.0                                                   | 3.0 | 4.1                                                       | 3.3  | 2.7                                                       | 4.2  | 7.9                                 | 106 | 0.2                                            | 5.55 | 2.11                                              | 5.55 | 2.11                                   |  |
| PHE201-07                | P      | 2.7                                                   | 2.4 | 3.7                                                       | 3.7  | 4.0                                                       | 4.3  | 8.6                                 | 26  | 0.1                                            | 6.83 | 2.46                                              | 6.83 | 2.46                                   |  |
| PHE201-08                | P      | 3.7                                                   | 3.3 | 4.6                                                       | 3.5  | 4.3                                                       | 5.9  | 10.5                                | 117 | 0.2                                            | 7.60 | 4.19                                              | 7.60 | 4.19                                   |  |
| PHE201-09                | S      | 3.7                                                   | 3.5 | 4.3                                                       | 3.7  | 3.5                                                       | 5.0  | 9.6                                 | 129 | 0.1                                            | 7.08 | 2.84                                              | 7.08 | 2.84                                   |  |
| PHE201-10                | P      | 1.5                                                   | 1.8 | 3.5                                                       | 1.6  | 1.9                                                       | 4.5  | 5.3                                 | 98  | 0.2                                            | 3.87 | 2.72                                              | 3.87 | 2.72                                   |  |
| PHE201-11                | P      | 0.4                                                   | 0.5 | 0.8                                                       | 0.4  | 0.4                                                       | 0.5  | 1.8                                 | 94  | 0.6                                            | 3.72 | 0.81                                              | 3.72 | 0.81                                   |  |
| PHE201-12                | P      | 7.3                                                   | 5.5 | 4.7                                                       | 6.7  | 7.8                                                       | 9.1  | 10.1                                | 15  | 0.2                                            | 3.62 | 4.71                                              | 3.62 | 4.71                                   |  |
| PHE201-13                | P      | 5.5                                                   | 4.4 | 4.7                                                       | 6.8  | 5.2                                                       | 7.3  | 10.4                                | 88  | 0.2                                            | 8.60 | 4.71                                              | 8.60 | 4.71                                   |  |
| PHE201-14                | P      | 5.8                                                   | 5.1 | 4.7                                                       | 5.6  | 6.3                                                       | 8.0  | 10.6                                | 96  | 0.2                                            | 4.44 | 3.42                                              | 4.44 | 3.42                                   |  |
| PHE201-15                | S      | 8.5                                                   | 8.1 | 4.7                                                       | 9.0  | 10.0                                                      | 10.8 | 11.2                                | 113 | 0.1                                            | 9.25 | 5.94                                              | 9.25 | 5.94                                   |  |
| PHE201-16                | P      | 9.1                                                   | 5.5 | 4.7                                                       | 8.3  | 8.4                                                       | 10.8 | 11.0                                | 103 | 0.2                                            | 9.77 | 5.16                                              | 9.77 | 5.16                                   |  |
| PHE201-17                | P      | 5.1                                                   | 5.0 | 4.7                                                       | 7.0  | 7.1                                                       | 8.9  | 10.9                                | 103 | 0.2                                            | 8.55 | 3.64                                              | 8.55 | 3.64                                   |  |
| PHE201-18                | S      | 1.8                                                   | 1.9 | 2.6                                                       | 1.7  | 2.0                                                       | 2.6  | 6.8                                 | 80  | 0.1                                            | 6.65 | 2.6                                               | 6.65 | 2.6                                    |  |
| PHE201-19                | S      | 3.9                                                   | 3.0 | 4.1                                                       | 3.0  | 3.0                                                       | 4.8  | 8.5                                 | 108 | 0.2                                            | 5.04 | 3.54                                              | 5.04 | 3.54                                   |  |
| PHE201-20                | S      | 2.2                                                   | 2.4 | 2.6                                                       | 2.2  | 2.7                                                       | 2.1  | 6.3                                 | 117 | 0.2                                            | 7.88 | 2.59                                              | 7.88 | 2.59                                   |  |
| PHE201-21                | P      | 3.1                                                   | 2.8 | 3.3                                                       | 2.7  | 2.5                                                       | 3.7  | 8.2                                 | 128 | 0.1                                            | 5.41 | 2.11                                              | 5.41 | 2.11                                   |  |
| PHE201-22                | P      | 2.5                                                   | 3.1 | 3.1                                                       | 2.6  | 2.4                                                       | 3.7  | 8.9                                 | 101 | 0.2                                            | 1.54 | 1.17                                              | 1.54 | 1.17                                   |  |
| PHE201-23                | P      | 2.1                                                   | 2.3 | 2.8                                                       | 2.8  | 2.7                                                       | 2.6  | 6.1                                 | 152 | 0.2                                            | 6.55 | 1.89                                              | 6.55 | 1.89                                   |  |
| PHE201-24                | P      | 3.8                                                   | 2.8 | 3.8                                                       | 2.5  | 3.4                                                       | 4.0  | 8.9                                 | 113 | 0.3                                            | 2.69 | 1.87                                              | 2.69 | 1.87                                   |  |
| PHE201-25                | P      | 2.7                                                   | 3.1 | 4.2                                                       | 2.9  | 3.3                                                       | 4.3  | 10.3                                | 114 | 0.2                                            | 9.13 | 4.89                                              | 9.13 | 4.89                                   |  |

TABLE 4

All panel members have been found positive by a test for HBsAg and negative by a test for anti-HIV-1. EIA and RIA results were generated using commercially available FDA approved anti-HBc IgM screening tests, performed at BBI and at a nationally recognized non-commercial reference laboratory (RL-1) by individuals who routinely use these procedures. All numeric results are means of duplicates, expressed as specimen absorbance to cutoff ratios (s/co). Ratios  $\geq 1.0$  are considered reactive. Specimens are undiluted aliquots from serum (S) or plasma (P) units collected from asymptomatic blood donors in 1989 and 1990.

**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE  
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS  
FOLLOWS:**

1. A recombinant protein useful in the diagnosis, treatment or prophylaxis of  
HIV comprising an amino acid sequence of HIV-1 gag protein p45, optionally  
fused to an amino acid sequence of a selected fusion partner protein, wherein the  
amino acid sequence of the HIV-1 gag protein p45 is as follows:

MetGlyAlaArgAlaSerValLeuSerGlyGlyGluLeuAspArgTrpGluLysIleArg  
LeuArgProGlyGlyLysLysLysTyrLysLeuLysHisIleValTrpAlaSerArgGlu  
LeuGluArgPheAlaValAsnProGlyLeuLeuGluThrSerGluGlyCysArgGlnIle  
LeuGlyGlnLeuGlnProSerLeuGlnThrGlySerGluGluLeuArgSerLeuTyrAsn  
ThrValAlaThrLeuTyrCysValHisGlnArgIleGluIleLysAspThrLysGluAla  
LeuAspLysIleGluGluGluGlnAsnLysSerLysLysLysAlaGlnGlnAlaAlaAla  
AspThrGlyHisSerAsnGlnValSerGlnAsnTyrProIleValGlnAsnIleGlnGly  
GlnMetValHisGlnAlaIleSerProArgThrLeuAsnAlaTrpValLysValValGlu  
GluLysAlaPheSerProGluValIleProMetPheSerAlaLeuSerGluGlyAlaThr  
ProGlnAspLeuAsnThrMetLeuAsnThrValGlyGlyHisGlnAlaAlaMetGlnMet  
LeuLysGluThrIleAsnGluGluAlaAlaGluTrpAspArgValHisProValHisAla  
GlyProIleAlaProGlyGlnMetArgGluProArgGlySerAspIleAlaGlyThrThr  
SerThrLeuGlnGluGlnIleGlyTrpMetThrAsnAsnProProIleProValGlyGlu  
IleTyrLysArgTrpIleIleLeuGlyLeuAsnLysIleValArgMetTyrSerProThr  
SerIleLeuAspIleArgGlnGlyProLysGluProPheArgAspTyrValAspArgPhe  
TyrLysThrLeuArgAlaGluGlnAlaSerGlnGluValLysAsnTrpMetThrGluThr  
LeuLeuValGlnAsnAlaAsnProAspCysLysThrIleLeuLysAlaLeuGlyProAla  
AlaThrLeuGluGluMetMetThrAlaCysGlnGlyValGlyGlyProGlyHisLysAla  
ArgValLeuAlaGluAlaMetSerGlnValThrAsnSerAlaThrIleMetMetGlnArg  
GlyAsnPheArgAsnGlnArgLysIleValLysCysPheAsnCysGlyLysGluGlyHis  
ThrAlaArgAsnCysArgAlaProArgLysLysGlyCysTrpLysCysGlyLysGluGly  
HisGlnMetLysAspCysThrGluArgGlnAlaAsnPheLeuGlyLysIle

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

2. A protein of claim 1 which is gag-gp41 A wherein the amino acid sequence  
5 of the fusion partner protein is as follows:

GlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArgAlaValGlyIle  
GlyAlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSerMet  
ThrLeuThrValGlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnAsnLeu  
10 LeuArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLysGln  
LeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeuGlyIle  
TrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAla

or any deletions, substitutions, inversions, insertions, additions or replacements  
15 encoding for a functional protein.

3. A protein of claim 1 which is gag-gp41 B wherein the amino acid sequence  
of the fusion partner protein is as follows:

20 ArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLysGlnLeu  
GlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeuGlyIleTrp  
GlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAlaSerTrpSerAsn  
LysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArgGluIleAsn  
AsnTyrThrSerLeuIleHisSerLeuIleGluGluSerGlnAsnGlnGlnGluLysAsn  
25 GluGlnGluLeuLeuGluLeuAspLysTrpAlaSerLeuTrp

or any deletions, substitutions, inversions, insertions, additions or replacements  
encoding for a functional protein.

4. A protein of claim 1 which is gag-gp41 C wherein the amino acid sequence  
30 of the fusion partner protein is as follows:

GlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArgAlaValGly  
 IleGlyAlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSer  
 MetThrLeuThrValGlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnAsn  
 LeuLeuArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLys  
 5 GlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeuGly  
 IleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAlaSerTrp  
 SerAsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArgGlu  
 IleAsnAsnTyrThrSerLeuIleHisSerLeuIleGluGluSerGlnAsnGlnGlnGlu  
 LysAsnGluGlnGluLeuLeuGluLeuAspLysTrpAlaSerLeuTrp

10

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

15

5. The protein of claim 1 wherein the fusion partner protein has at least one immunoreactive domain.

20

6. The protein of claim 1 wherein the said protein is expressed in a baculovirus system or an equivalent thereof.

7. The protein of claim 2 wherein the said protein is expressed in a baculovirus system or an equivalent thereof.

25

8. The protein of claim 3 wherein the said protein is expressed in a baculovirus system or an equivalent thereof.

9. The protein of claim 4 wherein the said protein is expressed in a baculovirus system or an equivalent thereof.

30

10. A DNA sequence useful in the diagnosis, treatment or prophylaxis of HIV encoding an HIV-1 gag protein p45, optionally fused to a sequence encoding a selected fusion partner protein, wherein the nucleotide sequence of the HIV-1

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

5

11. The DNA sequence of claim 10 wherein the fusion partner protein has at least one immunoreactive domain.

10

12. The DNA sequence of claim 10 wherein the nucleotide sequence of the fusion partner protein is as follows:

15

ggagtagca cccaccaagg  
caaagagaag agtgggtgcag agagaaaaaa gagcagtggg aataggagct ttgttccttg  
ggttcttggg agcagcagga agcactatgg gcgcagcgtc aatgacgctg acggtacagg  
ccagacaatt attgtctggt atagtgcagc agcagaacaa ttgctgagg gctattgagg  
cgcaacagca tctgttgcaa ctcacagtct ggggcatcaa gcagctccag gcaagaatcc  
tggctgtgga aagataccta aaggatcaac agctcctggg gatttggggt tgctctggaa  
aactcattg caccactgct gtgccttgga atgctTAA

20

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

25

13. The DNA sequence of claim 10 wherein the nucleotide sequence of the fusion partner protein is as follows:

30

agg gctattgagg  
cgcaacagca tctgttgcaa ctcacagtct ggggcatcaa gcagctccag gcaagaatcc  
tggctgtgga aagataccta aaggatcaac agctcctggg gatttggggt tgctctggaa  
aactcattg caccactgct gtgccttgga atgctagttg gagtaataaa tctctggaac  
agatttgga taacatgacc tggatggagt gggacagaga aattaacaat tacacaagct  
taatacactc ctttaattgaa gaatcgcaaa accagcaaga aaagaatgaa caagaattat  
tggaattaga taaatgggca agtttggtgTAA

## 33

gag protein p45 is as follows:

ATGGGTGCC

GAG CDS START—

AGAGCGTCAG TATTAAGCGG GGGAGAATTA GATCGATGGG AAAAAATTCG

5 GTTAAGGCCA GGGGGAAAGA AAAAATATAA ATTAAAACAT ATAGTATGGG

CAAGCAGGGA GCTAGAACGA TTCGCAGTTA ATCCTGGCCT GTTAGAAACA

TCAGAAGGCT GTAGACAAAT ACTGGGACAG CTACAACCAT CCCTTCAGAC

AGGATCAGAA GAACTTAGAT CATTATATAA TACAGTAGCA ACCCTCTATT

GTGTGCATCA AAGGATAGAG ATAAAAGACA CCAAGGAAGC TTAGACAAG

10 ATAGAGGAAG AGCAAAACAA AAGTAAGAAA AAAGCACAGC AAGCAGCAGC

P17 || P24

TGACACAGGA CACAGCAGTC AGGTCAGCCA AAATTACCCT ATAGTGCAGA

ACATCCAGGG GCAAATGGTA CATCAGGCCA TATCACCTAG AACTTTAAAT

GCATGGGTAA AAGTAGTAGA AGAGAAGGCT TTCAGCCCAG AAGTAATACC

CATGTTTTCA GCATTATCAG AAGGAGCCAC CCCACAAGAT TTAAACACCA

15 TGCTAAACAC AGTGGGGGGA CATCAAGCAG CCATGCAAAT GTTAAAAGAG

ACCATCAATG AGGAAGCTGC AGAATGGGAT AGAGTACATC CAGTGCATGC

AGGGCCTATT GCACCAGGCC AGATGAGAGA ACCAAGGGGA AGTGACATAG

CAGGAECTAC TAGTACCCTT CAGGAACAAA TAGGATGGAT GACAAATAAT

20 CCACCTATCC CAGTAGGAGA AATTTATAAA AGATGGATAA TCCTGGGATT

AAATAAAATA GTAAGAATGT ATAGCCCTAC CAGCATTCTG GACATAAGAC

AAGGACCAA AGAACCTTTT AGAGACTATG TAGACCGGTT CTATAAACT

CTAAGAGCCG AGCAAGCTTC ACAGGAGGTA AAAAATTGGA TGACAGAAAC

CTTGTTGGTC CAAAATGCCA ACCCAGATTG TAAGACTATT TTAAAAGCAT

25 TGGGACCAGC GGCTACACTA GAAGAAATGA TGACAGCATG TCAGGGAGTA

P24 | P15

GGAGGACCCG GCCATAAGGC AAGAGTTTTG GCTGAAGCAA TGAGCCAAGT

AACAAATACA GCTACCATAA TGATGCAGAG AGGCATTTT AGGAACCAA

GAAAGATGGT TAAGTGTTTC AATTGTGGCA AAGAAGGGCA CACAGCCAGA

AATTGCAGGG CCCCTAGGAA AAAGGGCTGT TGGAAATGTG GAAAGGAAGG

30 ACACCAAATG AAAGATTGTA CTGAGAGACA GGCTAATTTT TTAGGGAAGA

TCTAA

## 34

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

14. The DNA sequence of claim 10 wherein the nucleotide sequence of the fusion partner protein is as follows:

5 ggagtagca cccaccaagg  
caaagagaag agtgggtgcag agagaaaaaa gagcagtggg aataggagct ttgttccttg  
ggttcttggg agcagcagga agcactatgg gcgcagcgtc aatgacgtg acggtacagg  
ccagacaatt attgtctggt atagtgcagc agcagaacaa ttgctgagg gctattgagg  
10 cgcaacagca tctgttgcaa ctacagtct ggggcatcaa gcagctccag gcaagaatcc  
tggtctgtga aagataccta aaggatcaac agctcctggg gatttggggg tgctctggaa  
aactcattg caccactgct gtgccttgga atgctagtgt gagtaataaa tctctggaac  
agatttgga taacatgacc tggatggagt gggacagaga aattaacaat tacacaagct  
taatacactc attaattgaa gaatcgcaa accagcaaga aaagaatgaa caagaattat  
15 tggaattaga taaatgggca agtttgtggTAA

or any deletions, substitutions, inversions, insertions, additions or replacements encoding for a functional protein.

- 20 15. The use of an insoluble form of at least one recombinant protein as a carrier and antigen in a flow cytometric immunofluorescence assay for the detection of a disease state wherein said protein reacts with an antibody present in said disease state.

- 25 16. The use of claim 15 wherein the recombinant protein is expressed in a baculovirus system or an equivalent thereof.

17. The use of claim 15 wherein the disease state is selected from the group comprising HIV, hepatitis and HTLV.

30

18. The use of claim 17 wherein the disease state is selected from the

group comprising HIV-1, HIV-2, hepatitis B, hepatitis C, HTLV-1 and HTLV-2.

19. The use of claim 18 wherein the disease state is HIV-1.

5 20. The use of claim 18 wherein the disease state is hepatitis B.

21. The use of claim 17 wherein the disease state is HIV and wherein the protein is selected from the group comprising the proteins of any one of claims 1 to 9, pol97 and gp160.

10

22. The use of claim 21 wherein a mixture of gag-p45, pol97 and gp160 is utilized as carrier and antigen.

15

23. A method for the detection of a disease state which comprises utilizing an insoluble form of at least one recombinant protein as a carrier and antigen in a flow cytometric immunofluorescence assay for the detection of antibodies present in said disease state.

20

24. The method of claim 23 wherein the recombinant protein is expressed in a baculovirus system or an equivalent thereof.

25. The method of claim 23 wherein the disease state is selected from the group comprising HIV, hepatitis and HTLV.

25

26. The method of claim 25 wherein the disease is selected from the group comprising HIV-1, HIV-2, hepatitis B, hepatitis C, HTLV-1 and HTLV-2.

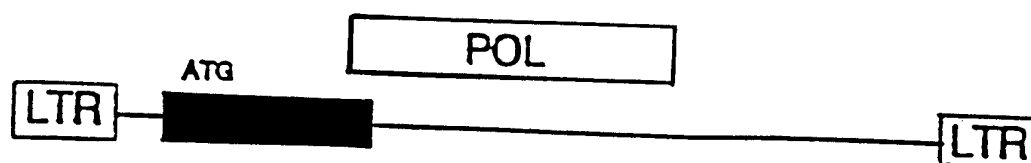
27. The method of claim 26 wherein the disease state is HIV-1.

30

28. The method of claim 26 wherein the disease state is hepatitis B.

29. The method of claim 25 wherein the disease state is HIV and wherein the protein is selected from the group comprising the proteins of any one of claims 1 to 9, pol97 and gp160.
- 5 30. The method of claim 29 wherein a mixture of gag-p45, pol97 and gp160 is utilized as carrier and antigen.

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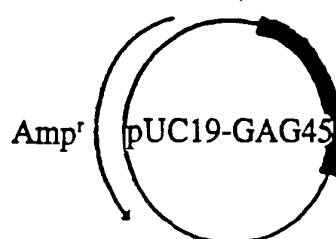
Isolation of ClaI-BglII fragment and insert into BamH site of pUC19 by ligation with linkers.

LINKER 1

BamH I

Cla I

GATCCATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGAT  
 GTACCCACGCTCTCGCAGTCATAATTCGCCCCCTCTTAATCTAGC



LINKER 2

Bgl II    BamHI    Bgl II

GATCTCTAAGGATCCTTA  
 AGATTCCTAGGAATCTAG

Figure 1A

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Isolation of Bam HI fragment

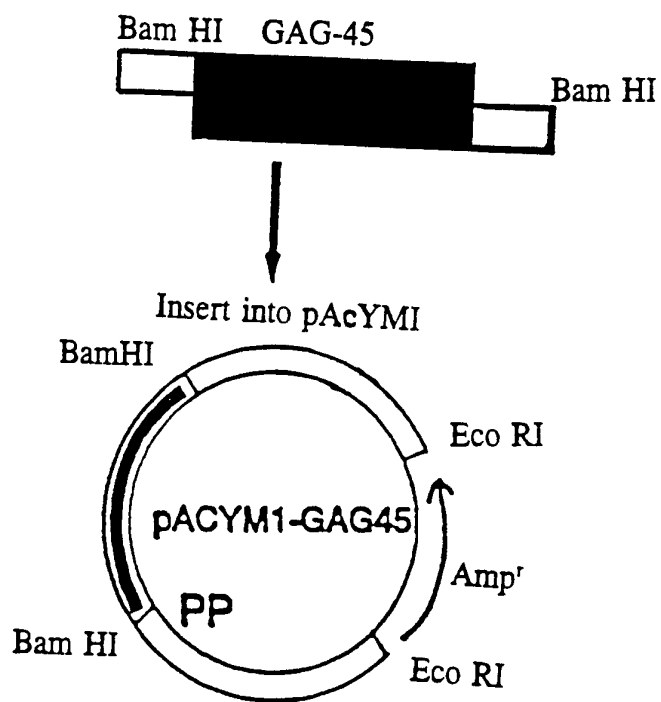


Figure 1B

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## Nucleotide Sequence of Truncated HIV-1 gag Polyprotein p45

|             |               |            |            |
|-------------|---------------|------------|------------|
|             |               |            | ATGGGTGCG  |
|             |               | GAG        | CDS START  |
| AGAGCGTCAG  | TATTAAGCGG    | GGGAGAATTA | GATCGATGGG |
| AAAAAATTCG  | GTTAAGGCCA    | GGGGGAAAGA | AAAAATATAA |
| ATTAAAACAT  | ATAGTATGGG    | CAAGCAGGGA | GCTAGAACGA |
| TTCGCAGTTA  | ATCCTGGCCT    | GTTAGAAACA | TCAGAAGGCT |
| GTAGACAAAT  | ACTGGGACAG    | CTACAACCAT | CCCTTCAGAC |
| AGGATCAGAA  | GAACCTAGAT    | CATTATATAA | TACAGTAGCA |
| ACCCTCTATT  | GTGTGCATCA    | AAGGATAGAG | ATAAAAGACA |
| CCAAGGAAGC  | TTAGACAAG     | ATAGAGGAAG | AGCAAAACAA |
| AAGTAAGAAA  | AAAGCACAGC    | AAGCAGCAGC | TGACACAGGA |
|             | ← P17   P24 → |            |            |
| CACAGCAGTC  | AGGTCAGCCA    | AAATTACCCT | ATAGTGCAGA |
| ACATCCAGGG  | GCAAATGGTA    | CATCAGGCCA | TATCACCTAG |
| AACCTTTAAAT | GCATGGGTAA    | AAGTAGTAGA | AGAGAAGGCT |
| TTCAGCCCAG  | AAGTAATACC    | CATGTTTTCA | GCATTATCAG |
| AAGGAGCCAC  | CCCACAAGAT    | TTAAACACCA | TGCTAAACAC |
| AGTGGGGGGA  | CATCAAGCAG    | CCATGCAAAT | GTAAAAAGAG |
| ACCATCAATG  | AGGAAGCTGC    | AGAATGGGAT | AGAGTACATC |
| CAGTGCATGC  | AGGGCCTATT    | GCACCAGGCC | AGATGAGAGA |
| ACCAAGGGGA  | AGTGACATAG    | CAGGAACTAC | TAGTACCCTT |
| AAGGAACAAA  | TAGGATGGAT    | GACAAATAAT | CCACCTATCC |
| CAGTAGGAGA  | AATTTATAAA    | AGATGGATAA | TCCTGGGATT |
| AAATAAAATA  | GTAAGAATGT    | ATAGCCCTAC | CAGCATTCTG |
| GACATAAGAC  | AAGGACCAAA    | AGAACCTTTT | AGAGACTATG |
| TAGACCGGTT  | CTATAAACT     | CTAAGAGCCG | AGCAAGCTTC |
| ACAGGAGGTA  | AAAAATTGGA    | TGACAGAAAC | CTTGTTGGTC |
| CAAAATGCGA  | ACCCAGATTG    | TAAGACTATT | TTAAAAGCAT |

Figure 2A

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|            |            |                   |                   |
|------------|------------|-------------------|-------------------|
| TGGGACCAGC | GGCTACACTA | GAAGAAATGA<br>P24 | TGACAGCATG<br>P15 |
| TCAGGGAGTA | GGAGGACCCG | GCCATAAGGC        | AAGAGTTTTG        |
| GCTGAAGCAA | TGAGCCAAGT | AACAAATACA        | GCTACCATAA        |
| TGATGCAGAG | AGGCAATTTT | AGGAACCAAA        | GAAAGATGGT        |
| TAAGTGTTTC | AATTGTGGCA | AAGAAGGGCA        | CACAGCCAGA        |
| AATTGCAGGG | CCCCTAGGAA | AAAGGGCTGT        | TGGAAATGTG        |
| GAAAGGAAGG | ACACCAAATG | AAAGATTGTA        | CTGAGAGACA        |
| GGCTAATTTT | TTAGGGAAGA | TCTAA             |                   |

Figure 2B

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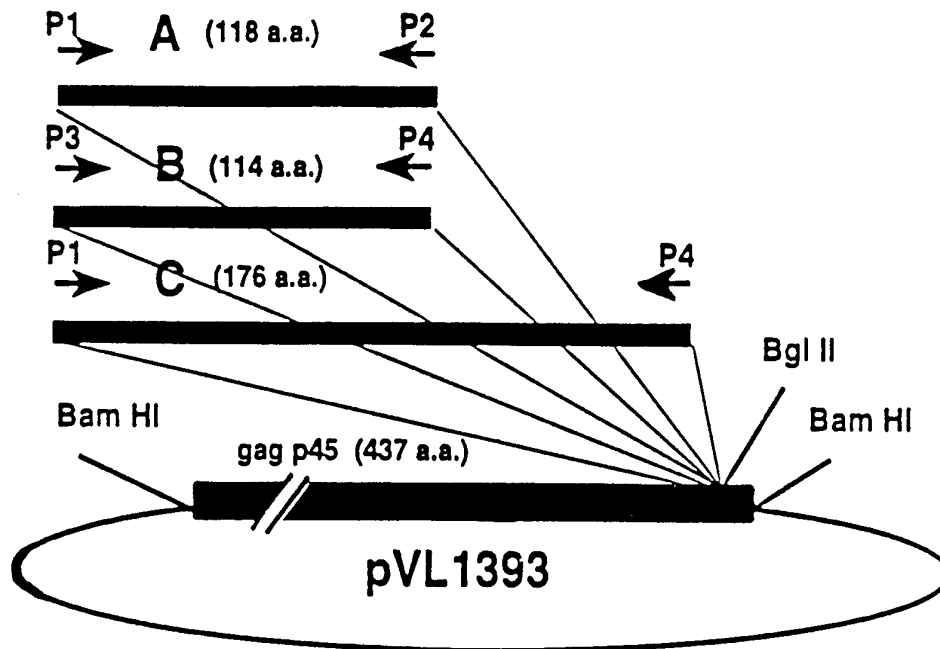
## Amino Acid Sequence of HIV-1 gag Protein p45

MetGlyAlaArgAlaSerValLeuSerGlyGlyGluLeuAspArgTrpGlu  
LysIleArgLeuArgProGlyGlyLysLysLysTyrLysLeuLysHisIle  
ValTrpAlaSerArgGluLeuGluArgPheAlaValAsnProGlyLeuLeu  
GluThrSerGluGlyCysArgGlnIleLeuGlyGlnLeuGlnProSerLeu  
GlnThrGlySerGluGluLeuArgSerLeuTyrAsnThrValAlaThrLeu  
TyrCysValHisGlnArgIleGluIleLysAspThrLysGluAlaLeuAsp  
LysIleGluGluGluGlnAsnLysSerLysLysLysAlaGlnGlnAlaAla  
AlaAspThrGlyHisSerAsnGlnValSerGlnAsnTyrProIleValGln  
AsnIleGlnGlyGlnMetValHisGlnAlaIleSerProArgThrLeuAsn  
AlaTrpValLysValValGluGluLysAlaPheSerProGluValIlePro  
MetPheSerAlaLeuSerGluGlyAlaThrProGlnAspLeuAsnThrMet  
LeuAsnThrValGlyGlyHisGlnAlaAlaMetGlnMetLeuLysGluThr  
IleAsnGluGluAlaAlaGluTrpAspArgValHisProValHisAlaGly  
ProIleAlaProGlyGlnMetArgGluProArgGlySerAspIleAlaGly  
ThrThrSerThrLeuGlnGluGlnIleGlyTrpMetThrAsnAsnProPro  
IleProValGlyGluIleTyrLysArgTrpIleIleLeuGlyLeuAsnLys  
IleValArgMetTyrSerProThrSerIleLeuAspIleArgGlnGlyPro  
LysGluProPheArgAspTyrValAspArgPheTyrLysThrLeuArgAla  
GluGlnAlaSerGlnGluValLysAsnTrpMetThrGluThrLeuLeuVal  
GlnAsnAlaAsnProAspCysLysThrIleLeuLysAlaLeuGlyProAla  
AlaThrLeuGluGluMetMetThrAlaCysGlnGlyValGlyGlyProGly  
HisLysAlaArgValLeuAlaGluAlaMetSerGlnValThrAsnSerAla  
ThrIleMetMetGlnArgGlyAsnPheArgAsnGlnArgLysIleValLys  
CysPheAsnCysGlyLysGluGlyHisThrAlaArgAsnCysArgAlaPro  
ArgLysLysGlyCysTrpLysCysGlyLysGluGlyHisGlnMetLysAsp  
CysThrGluArgGlnAlaAsnPheLeuGlyLysIle

Figure 3

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P1 5' CAG ATC TCC GGA GTA GCA CCC ACC  
 p2 5' GAG ATC TGT TAA GCA TTC CAA GGC AC  
 p3 5' CTC GAA GAT CTC CAG GGC TAT TGA GGC GCA  
 p4 5' CTC GAA GAT CTA TTA CCA CAA ACT TGC CCA

Figure 4

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## Nucleotide Sequence of Chimeric Protein A

gag-p45      GGAGTAGCACCCACCAAGGCAAGAGAGAGTGGTGACAGAGAGAAAAAGACAGTGGGAATA  
                  → +    GlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArgAlaValGlyIle  
 GGAGCTTTGTTCCCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAG  
 GlyAlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSerMetThrLeuThrValGln  
 GCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAACAATTGTGCTGAGGGCTATTGAGGCGCAACAGCATCTG  
 AlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnLeuLeuArgAlaIleGluAlaGlnGlnHisLeu  
 TTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA  
 LeuGlnLeuThrValTrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGln  
 CAGCTCCTGGGGATTGGGGTTGCTCTGGAAAACTCATTTGCACCACCTGCTGTGCCTTGGAAATGCT  
 GlnLeuLeuGlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAla

Figure 5

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## Nucleotide Sequence of Chimeric Protein B

gag-p45      AGGGCTATTGAGGCGCAACAGCATCTG  
                  → + ArgAlaIleGluAlaGlnHisLeu  
  
 TTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAATCCTGGCTGTGAAAGATACCTAAAGGATCAA  
 LeuGlnLeuThrValTrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGln  
  
 CAGCTCCTGGGGATTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCCTTGGAAATGCTAGTTGGAGT  
 GlnLeuLeuGlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAlaSerTrpSer  
  
 AATAATCTCTGGAACAGATTTGGAAATAACATGACCTGGATGGAGTGGGACAGAGAAATTAAACAATTACACAAGC  
 AsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArgGluIleAsnAsnTyrThrSer  
  
 TTAATACACTCCTTAATTGAAGAAATCGCAAAACCAAGCAAGAAAGAAATGAACAAGAAATTATTGGAAATTAGATAAA  
 LeuIleHisSerLeuIleGluGluSerGlnAsnGlnGlnGluLysAsnGluGlnGluLeuLeuGluLeuAspLys  
  
 TGGGCAAGTTTGTGG  
 TrpAlaSerLeuTrp

Figure 6



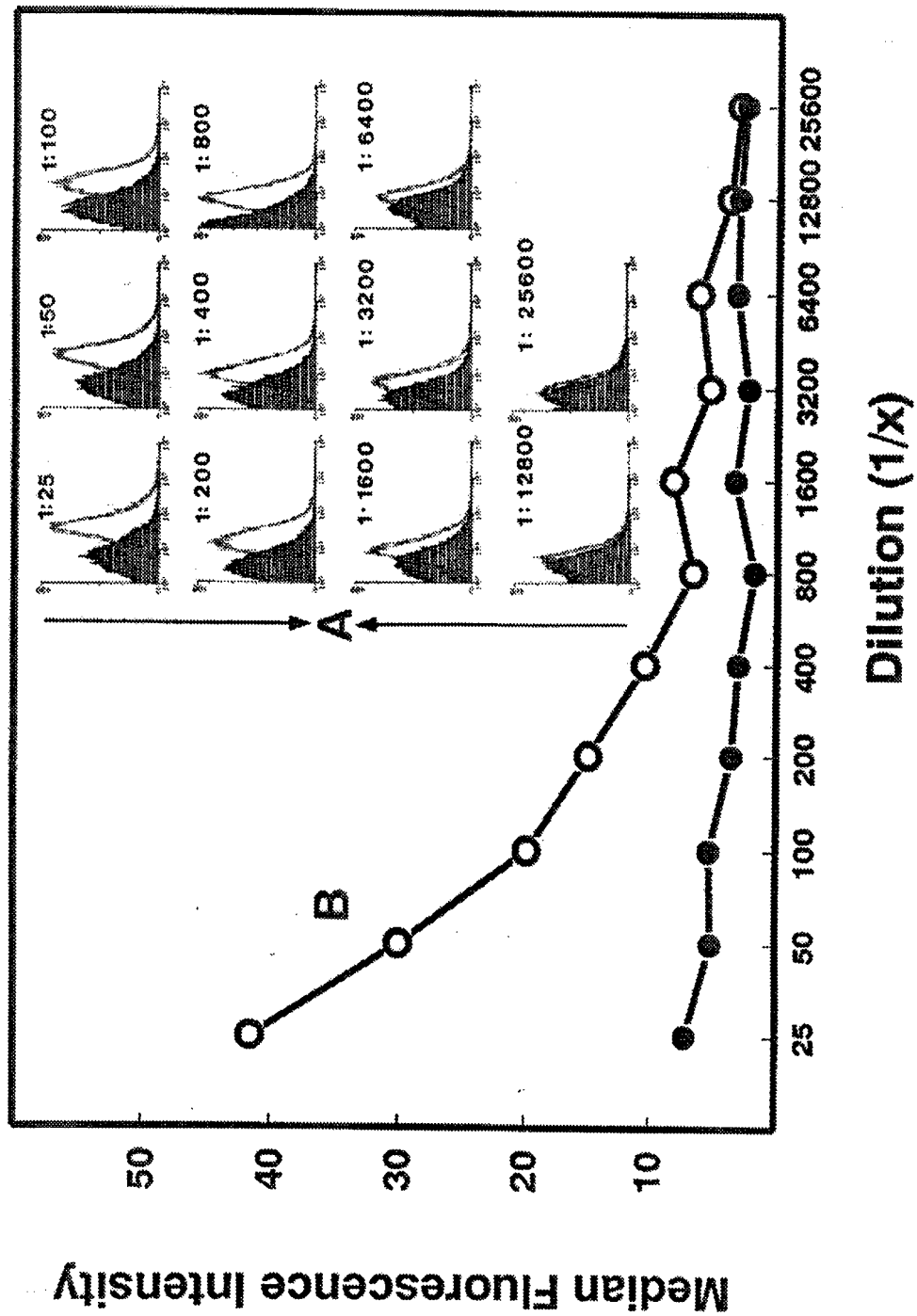


Figure 8A

SUBSTITUTE SHEET

11/22

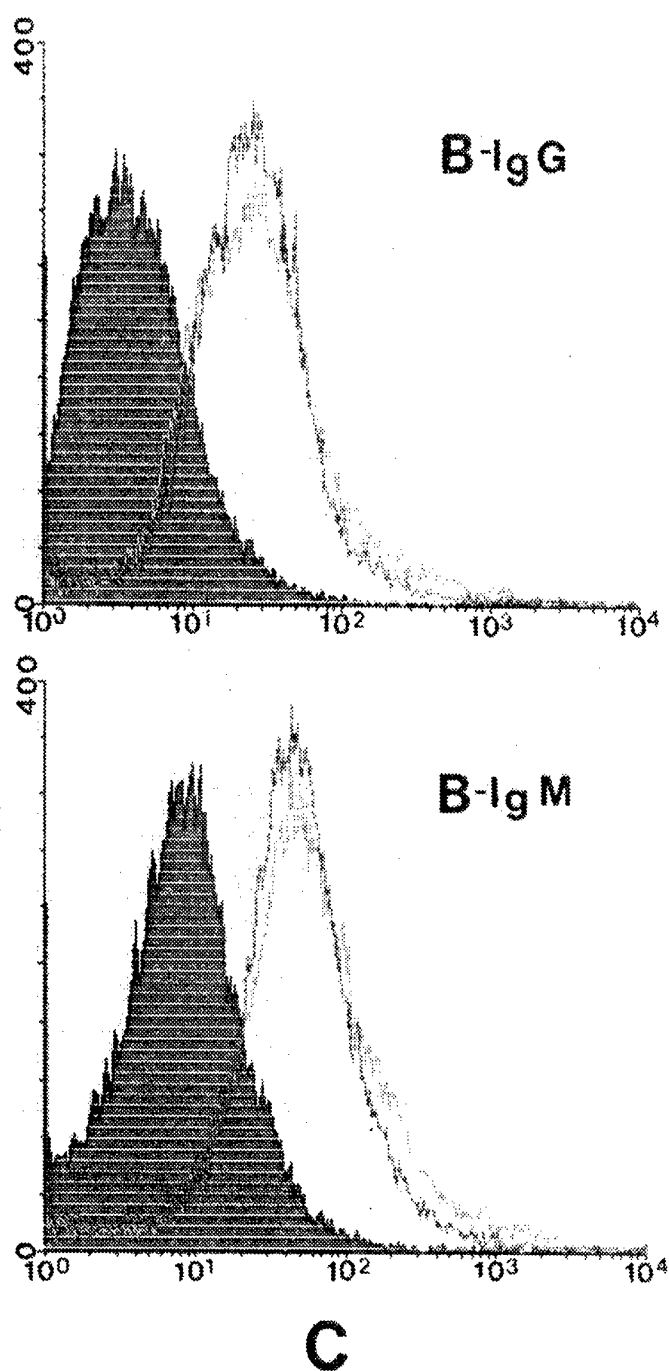


Figure 8B

SUBSTITUTE SHEET

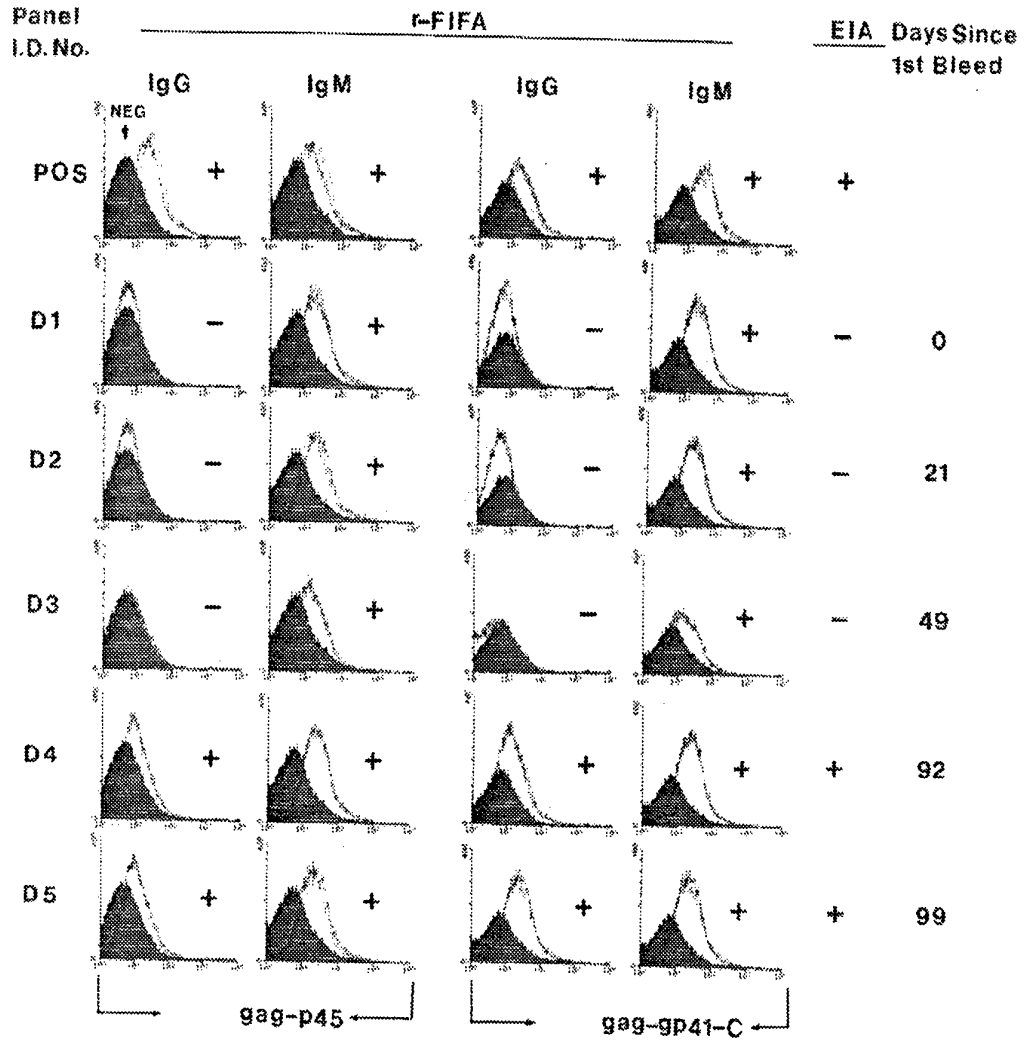


Figure 9

SUBSTITUTE SHEET

13/22

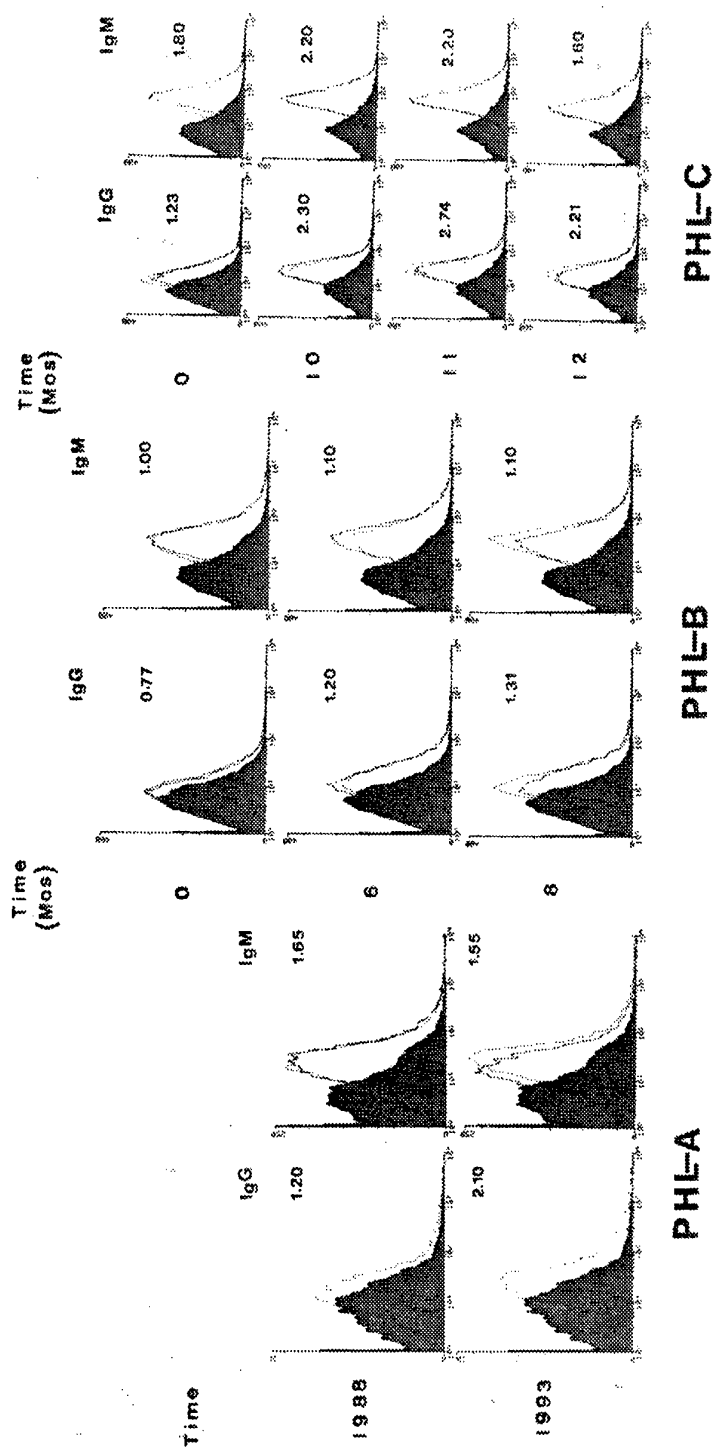


Figure 10

SUBSTITUTE SHEET

14/22

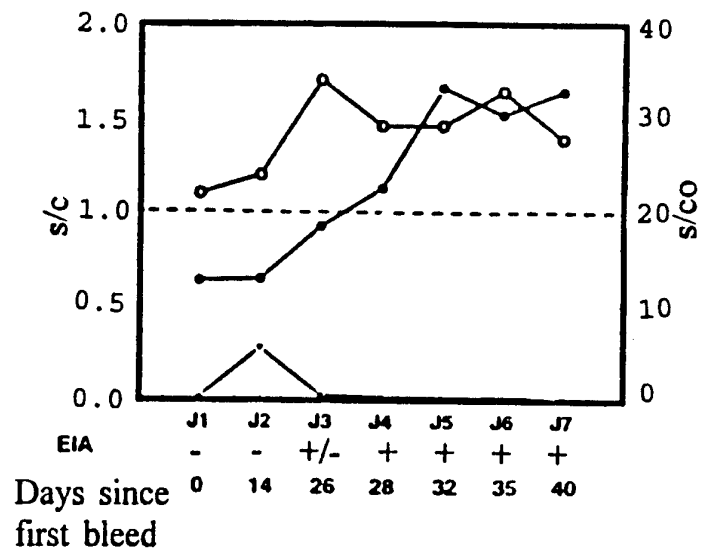
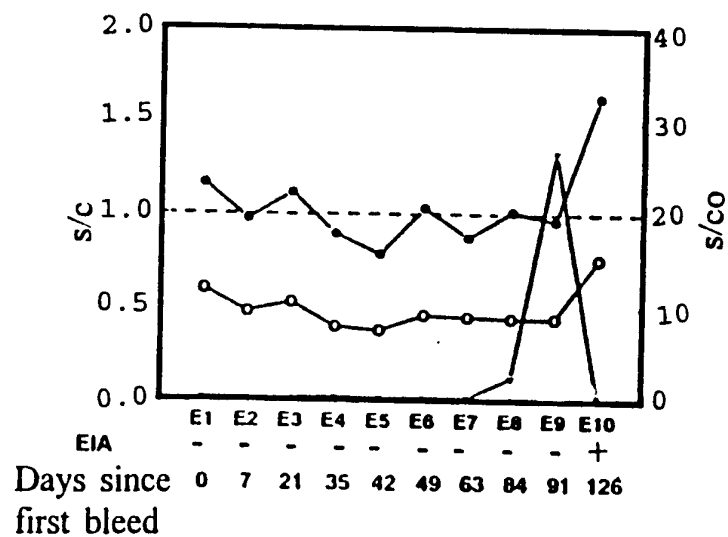
**A****B**

Figure 11A

SUBSTITUTE SHEET

15/22

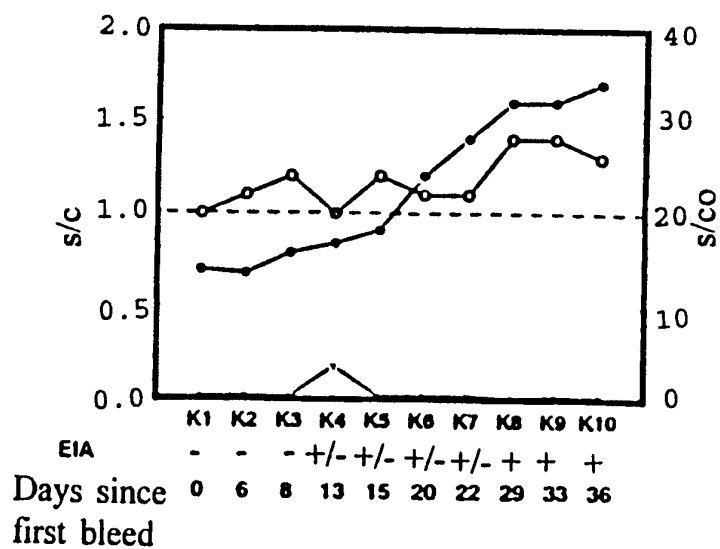
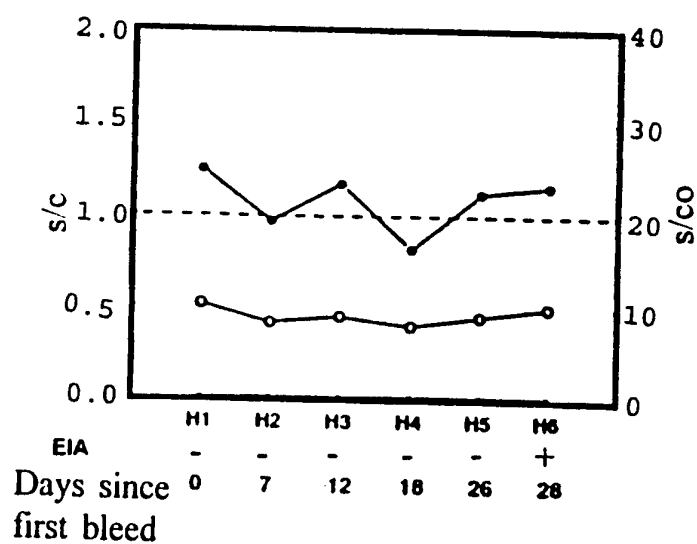
**A****B**

Figure 11B  
SUBSTITUTE SHEET

16/22

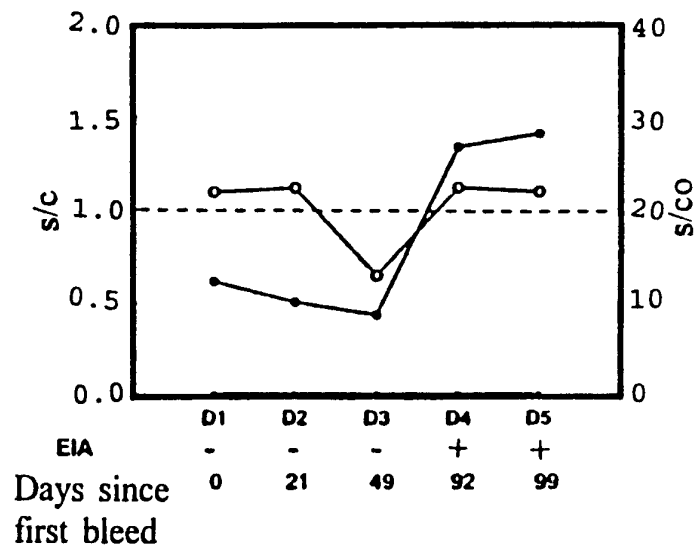
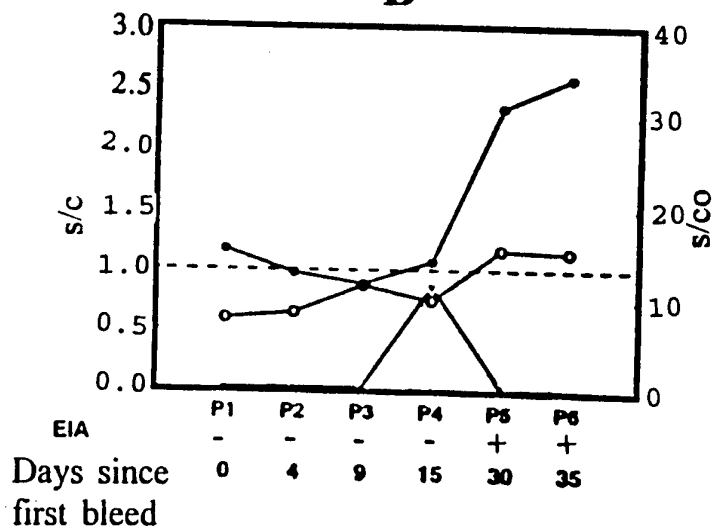
**A****B**

Figure 11C

SUBSTITUTE SHEET

17/22

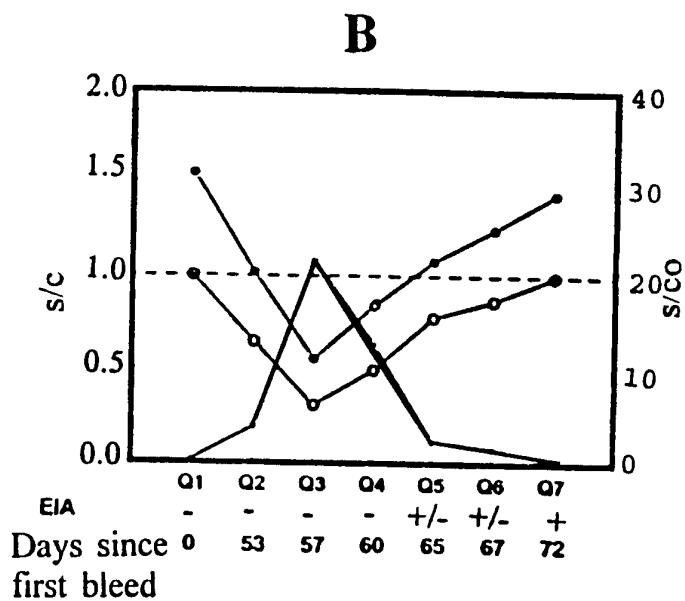
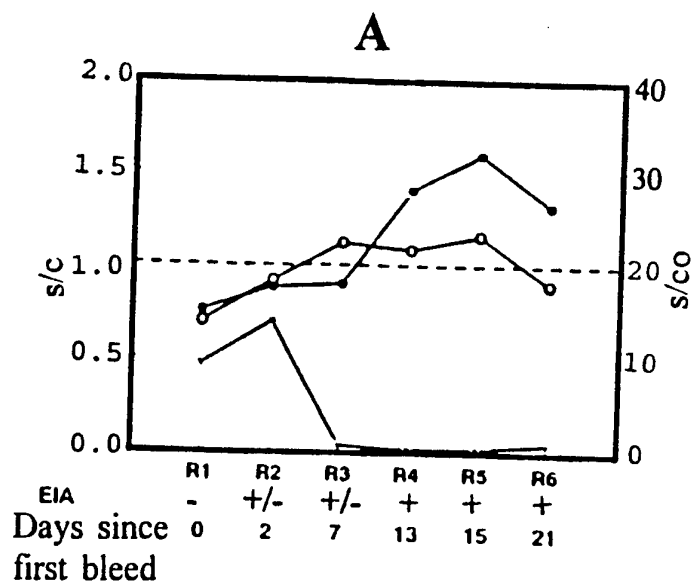


Figure 11D

SUBSTITUTE SHEET

18/22

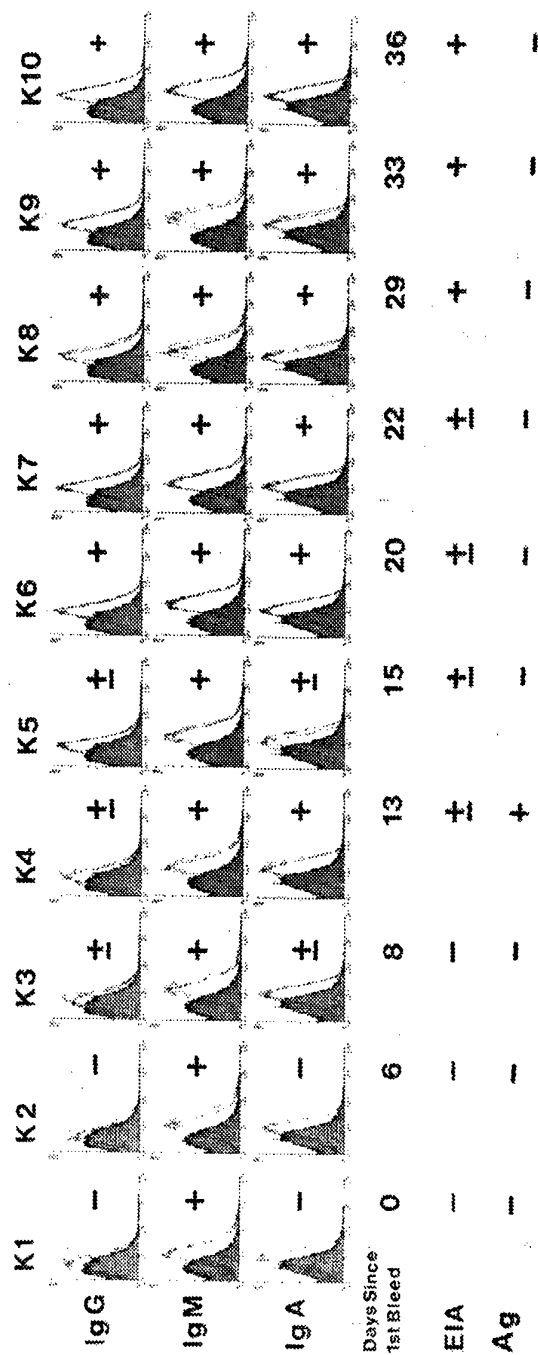


Figure 12

SUBSTITUTE SHEET

19/22

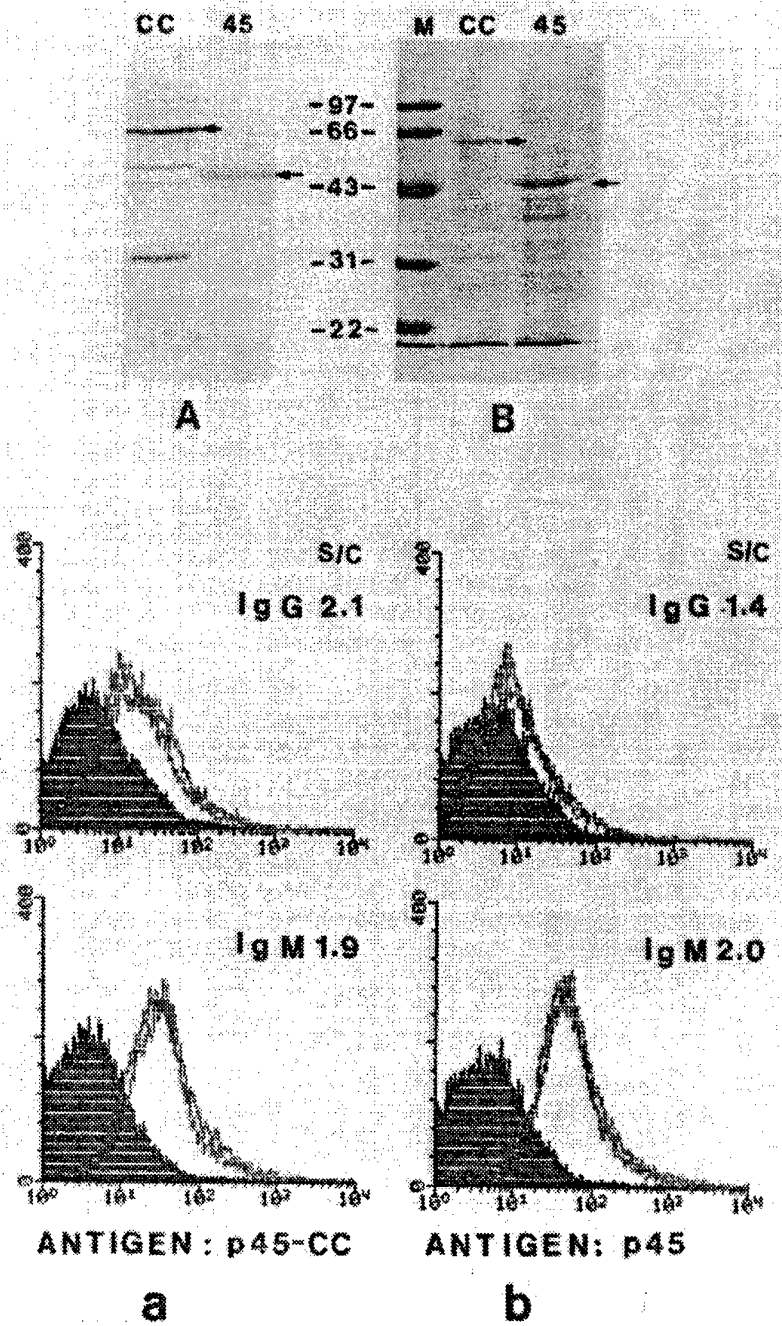


Figure 13

SUBSTITUTE SHEET

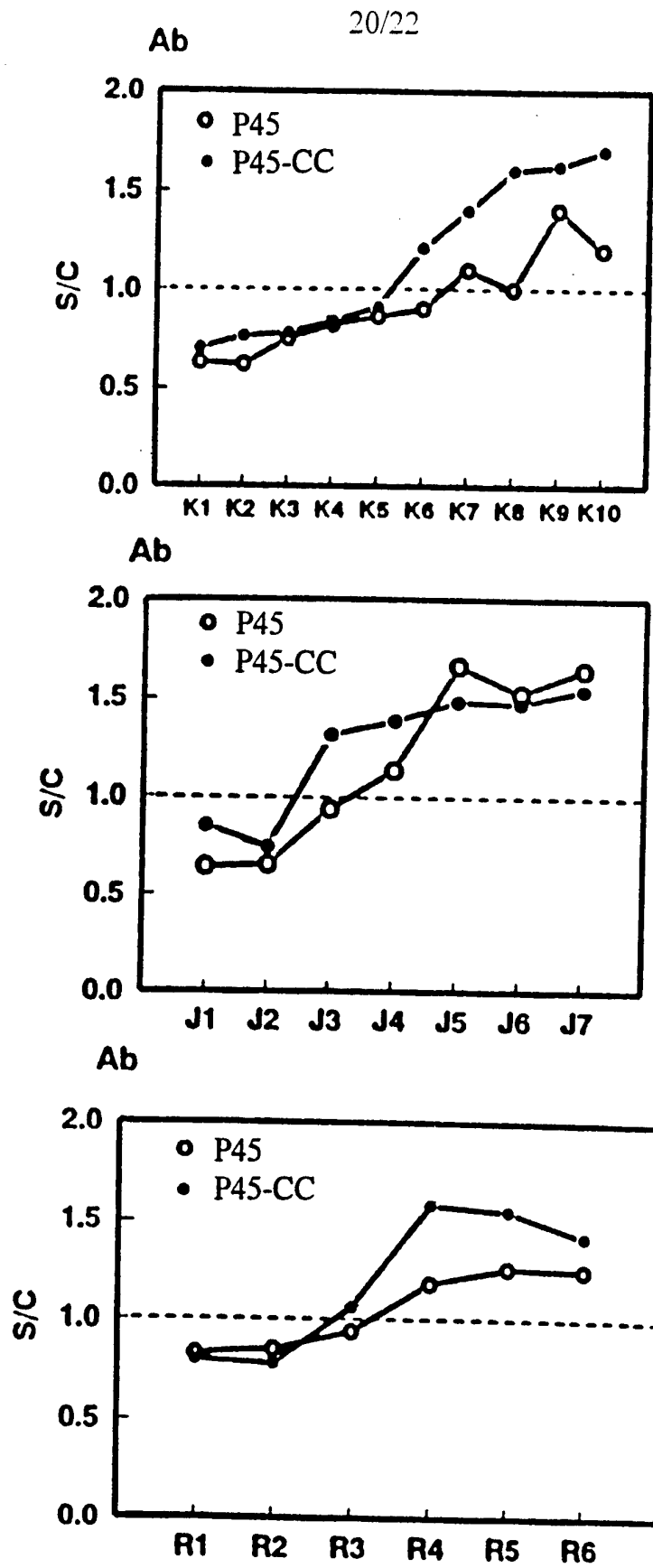


Figure 14  
SUBSTITUTE SHEET

21/22

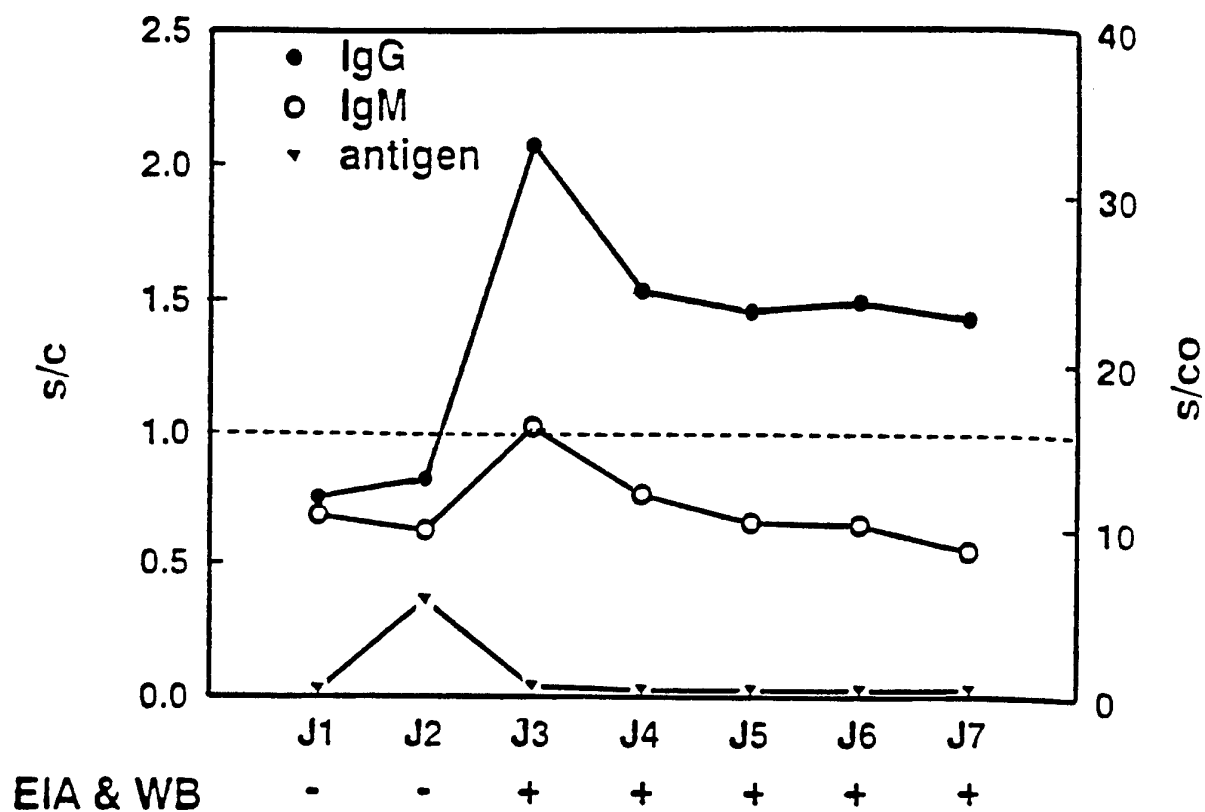


Figure 15

SUBSTITUTE SHEET

22/22

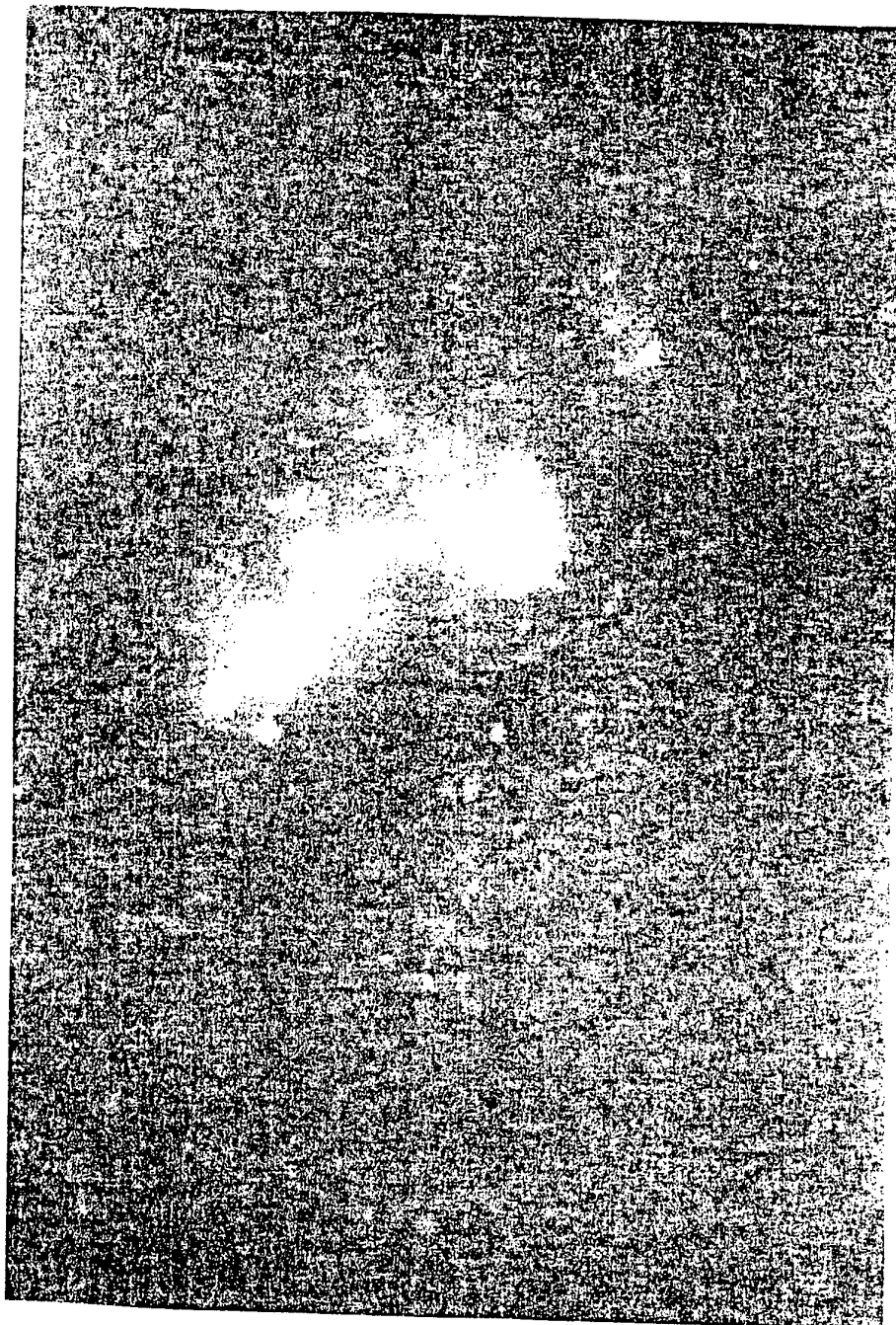


Figure 16

SUBSTITUTE SHEET