AU9058355

(12) PATENT ABRIDGMENT (11) Document No. AU-B-58355/90
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 642886

(54) Title
T-LYMPHOTROPIC RETROVIRUS MONOCLONAL ANTIBODIES

International Patent Classification(s)
(51)[5] **C12P 021/08** **C07K 007/06** **C07K 007/08** **C07K 007/10**
G01N 033/571

(21) Application No. : **58355/90** (22) Application Date : **14.05.90**

(87) PCT Publication Number : **WO90/14358**

(30) Priority Data

(31) Number	(32) Date	(33) Country
351882	**15.05.89**	**US UNITED STATES OF AMERICA**

(43) Publication Date : **18.12.90**

(44) Publication Date of Accepted Application : **04.11.93**

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(56) Prior Art Documents
AU 89766/91 C07K 7/06
AU 55765/90 G01N 33/571
AU 30761/89 C07K 7/06

(57) Claim

1. A monoclonal antibody which reacts with an epitope of p24 of HIV-1 and p26 of HIV-2, said epitope located within amino acid residues 140-160 of p24.

8. An epitope consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function and with which the monoclonal antibody of claim 1 reacts.

24. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 8 and measuring the formation of antigen-antibody complexes.

OPI DATE 18/12/90 APPLN. ID 58355 / 90

PCT AOJP DATE 07/02/91 PCT NUMBER PCT/US90/02874

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 7/06, 7/08, 15/28
G01N 33/543, 33/569, 33/571
G01N 33/577

A1

(11) International Publication Number: WO 90/14358

(43) International Publication Date: 29 November 1990 (29.11.90)

(21) International Application Number: PCT/US90/02874

(22) International Filing Date: 14 May 1990 (14.05.90)

(30) Priority data:
351,882 15 May 1989 (15.05.89) US

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

642886

(54) Title: T-LYMPHOTROPIC RETROVIRUS MONOCLONAL ANTIBODIES

(57) Abstract

The instant invention relates to monoclonal antibodies, the cell lines producing those antibodies, the peptides that comprise the epitopes of those antibodies and assays using those antibodies and peptides for the detection of HIV-1 and HIV-2 gene products. In particular, the antibodies react with the p24/p26 capsid protein, the nonapeptide that comprises an HIV-1/HIV-2 conserved epitope is disclosed and a capture ELISA using a combination of three monoclonal antibodies that can detect simultaneously HIV-1 and HIV-2 is disclosed.

TITLE OF THE INVENTION

T-LYMPHOTROPIC RETROVIRUS MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

The invention relates to monoclonal antibodies, peptides that comprise the epitopes of said monoclonal antibodies and assays utilizing said monoclonal antibodies and said peptides for the detection of T-lymphotropic retroviruses, particularly HIV-1, HIV-2 and SIV.

BACKGROUND OF THE INVENTION

The T-lymphotropic retrovirus family includes among other lentiviruses the simian retrovirus SIV and the human retroviruses HIV-1 (the likely etiologic agent of AIDS) and HIV-2. Although HIV-1 and HIV-2 are related evolutionally, nucleic acid sequence analysis reveals that HIV-2 is more closely related to SIV than it is to HIV-1. Guyader et al. (1987) noted only 42% overall genomic sequence identity between the HIV-1 and HIV-2 isolates they compared. Patients infected with HIV-2 can manifest disorders that typify AIDS, purely neurologic disease or asymptomatic infections (Kuhnel et al., 1988) despite HIV-1-related ultrastructural and biological properties such as *in vitro* cytopathogenicity and CD4 tropism (Clavel et al., 1986).

The HIV-1 and HIV-2 genomes have a typical retroviral configuration comprising LTR's, *gag* and *env* regions that encode viral structural proteins, sequences encoding one or more enzyme, including a reverse transcriptase and other ORF's and regulatory elements. The *gag* region of HIV-1 encodes a precursor peptide

known as p55. p55 is processed to produce among other proteins the major core or capsid protein known as p24. In HIV-2, the analogous gag precursor is larger, known as p57, and the major core protein is known as p26. Although a high degree of conservation of the gag proteins of HIV-1 and HIV-2 was expected, Guyader et al. (1987) found only 58% identity of amino acids between HIV-1 and HIV-2 gag proteins. Even among distant isolates of HIV-1 there is a greater than 90% identity of gag proteins. That and other data support the hypothesis that although HIV-1 and HIV-2 are somewhat related, they are nevertheless distinct retroviral species.

Because HIV-1 and possibly HIV-2 have such an impact on the human immune system, it is desirable, in fact imperative that sensitive, rapid diagnostic assays for detecting presence of HIV be available for population screening, quality control in blood banks, diagnosis, furtherance of our understanding of those viruses to assure the goal of obtaining a vaccine and cure, and the like. Because of ease and convenience, it is preferable that the assays be immunology-based, such as ELISA's, and for reproducibility, specificity and consistency that the reagents be monoclonal antibodies and defined antigenic peptides. Because p24 antigenemia has been shown to be an early sign of HIV infection (Kessler et al., 1987; Wall et al., 1987) and the observation that clinical progression of AIDS sequelae is associated with reduction in anti-p24 while patients with AIDS can die with high levels of anti-env titers (Coates et al., 1987), it would be advantageous for the assay to be directed to detecting gag products such as p24/p26.

Weiss et al. (1988) identified human serum samples that contained antibodies specific to HIV-2 gp130 in radioimmunoprecipitation assays and in ELISA's. Those

antibodies showed low level HIV-1 crossreactivity in a VSV pseudotype neutralization assay and in a neutralization of C8166 syncytia formation assay.

Minassian et al. described a monoclonal antibody identified as R1C7 that was raised against HIV-2. R1C7, an anti-capsid antibody (p26), reacted not only with the three HIV-2 isolates tested, but with the five HIV-1 isolates and seven SIV isolates that were tested. In immunoblots, R1C7 bound to 55KD and 26KD HIV-2 proteins, to 24KD and 55KD HIV-1 proteins and to a 28KD SIV protein.

Niedrig et al. developed a panel of 29 monoclonal antibodies to HIV-1. One antibody was directed to p17 and its precursor p32 whereas the remainder reacted with p24 and some of those also reacted with p55. The p17 antibody was found to be HIV-1 specific. Of the 28 anti-p24 antibodies, 20 reacted in immunoblots with the corresponding capsid protein (p26) of HIV-2 and five of those also recognized the corresponding SIV protein, p28. Niedrig et al. make no mention of antibody titer, the efficacy of the antibodies in a antigen capture assay or which of the antibodies bind to p26, p55 or both. Furthermore, several of antibodies reacted with a 22KD protein of unknown function in HIV-2 preparations.

Many diagnostic kits and assays have been developed for the detection of HIV-1 in samples of sera, blood, blood products or other body tissues. The assays use a variety of techniques such as Western blot, enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescent assay and employ either antibodies to whole virus or purified viral antigens, see for example, Gallo et al., U.S. Patent No. 4,520,113; Sarngadharan, et al., (1984); and Robert-Guroff et al. (1982).

Summary Of The Invention

The instant invention relates to monoclonal antibodies, the cell lines producing those antibodies, the peptides that comprise the epitopes of those antibodies and assays using those antibodies and peptides for the detection of HIV-1 and HIV-2 gene products as well as SIV gene products. In particular, the antibodies react with the p24/p26 capsid protein. The nonapeptide that comprises an HIV-1/HIV-2 conserved epitope is disclosed and a capture ELISA using a combination of three monoclonal antibodies that can detect simultaneously HIV-1 and HIV-2 is disclosed.

According to a first embodiment of the invention there is provided a monoclonal antibody which reacts with an epitope of p24 of HIV-1 and p26 of HIV-2, said epitope located within amino acid residues 140-160 of p24.

According to a second embodiment of the invention there is provided a monoclonal antibody which reacts with an antigen consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

According to a third embodiment of the invention there is provided a monoclonal antibody which reacts with an antigen consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

According to a fourth embodiment of the invention there is provided an epitope consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function and with which the monoclonal antibody of the first embodiment reacts.

According to a fifth embodiment of the invention there is provided an epitope consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys with which the monoclonal antibody of the first embodiment reacts.

According to a sixth embodiment of the invention there is provided an amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

According to a seventh embodiment of the invention there is provided an amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

According to an eighth embodiment of the invention there is provided a diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which reacts with an antigen of HIV-1 and a monoclonal antibody of the first, second or third embodiment.

According to a ninth embodiment of the invention there is provided a method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of the first, second or third embodiment, and measuring the formation of antigen-antibody complexes.

According to a tenth embodiment of the invention there is provided a method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of the fourth or fifth embodiment and measuring the formation of antigen-antibody complexes.

According to an eleventh embodiment of the invention there is provided a method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the amino acid sequence of the sixth or seventh embodiment and measuring the formation of antigen-antibody complexes.

According to a twelfth embodiment of the invention there is provided a diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample consisting of the epitope of the fourth or fifth embodiment.

According to a thirteenth embodiment of the invention there is provided a diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the amino acid sequence of the sixth or seventh embodiment.

Brief Description Of The Figures

Figure 1. Graph depicting reactivity of culture supernatants in capture ELISA. A detailed legend appears in Table 1.

Figure 2. Photograph of immunoblot nitrocellulose strips determining the specificity of anti-HIV antibodies.

Figure 3. Protein A-purified antibodies were used as probe to separated HIV-2 proteins in immunoblots. Lanes 1 and 2 are positive controls and Lane 3 is a negative control.

Figure 4. Diagram of some of the recombinant p24 peptides used to map epitopes.

Figure 5. Diagram of four regions of p24 to which various monoclonal antibodies bind.

Figure 6. Photographs of Westerns reacting various monoclonals with blotted gag and gag fragments. Lane 1 in each photo contains whole virus lysate. Lane 5 in each photo is a negative control $p24^-$ plasmid and Lane 6 in each photo is another negative control containing non-HIV-infected MOLT lysate.

Figure 7. Graph representing results of ELISA's using sequential overlapping nonapeptides as antigen to determine epitope of 7-D4.

Figure 8. Diagram depicting epitope mapping using sequential overlapping nonapeptides as antigen in ELISA.

Figure 9. Composition of the regions that comprise the 7-D4 epitope.

Figure 10. Graph of sensitivity of a capture ELISA using two anti-p24 antibodies, 6-C10 and 5-B4, on the solid phase and HIV-1 infected MOLT 3 lysate as the antigen. An HRP conjugated human anti-HIV was the reporter.

Figure 11. Graph of sensitivity of a capture ELISA using 6-C10 and 5-B4 with and without 7-D4 on the solid phase to detect p26 of HIV-2.

Figure 12. Dose response curve for HIV-1 and HIV-2 in a capture ELISA using 6-C10, 5-B4 and 7-D4.

Figure 13. Comparison of HIV-1 dose response curves between the three antibody capture ELISA and a reverse transcriptase assay.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention relates to monoclonal antibodies and their production, immunoassays and oligopeptides. The methods that were used are known in the art and are discussed only briefly throughout the specification. Suitable methods to practice the invention may be found in Meth Enzymology 121, (1986) and other available reference materials.

EXAMPLE:

Preparation of Monoclonal Antibodies

Monoclonal antibodies were produced according to established procedures (Kohler & Milstein, 1975). Briefly, female BALB/c mice were immunized

intraperitoneally repeatedly with lysates of HIV-1 infected MOLT 3 cells emulsified in complete Freund's adjuvant (50%). Sensitized spleen cells were fused with P3X63-Ag8.653 myeloma cells using PEG 1500. Heterokaryons were selected in HAT medium, cloned and screened for reactivity to HIV antigens in a capture ELISA. The IgG fraction of polyclonal human anti-HIV was coated onto wells of microtiter dishes. HIV-1 (produced in MOLT 3 cells) and culture supernatants were added simultaneously to the wells. Bound murine antibodies were detected with an enzyme-labelled anti-mouse Ig antibody. Data representative of the screening is depicted in Figure 1. Designation of the sample numbers is set forth in Table 1.

Table 1

ELISA Screening of Fusion F86

Sample No.	Designation
1	5-B4
2	5-D9
3	5-E2
4	5-F12
5	6-B9
6	4-E6
7	6-C10
8	6-E11
9	6-F6
10	10-B2
11	10-C12
12	10-D1
13	2-C8
14	7-D4
15	7-E1
16	7-E10

Table 1

ELISA Screening of Fusion F86 - (cont'd)

Sample No.	Designation
17	7-F3
18	8-E7
19	9-B7
20	9-D5
21	F86 Bleedout*
22	NMS**
23	Negative Control

* Serum obtained at sacrifice

** Normal Mouse Serum

Western Blots

Candidate anti-HIV clones were tested further in Western blots (Towbin et al., 1979). Lysates of HIV-infected MOLT 3 cells were separated through a 12% acrylamide gel under denaturing conditions. The proteins were transferred to nitrocellulose and individual strips were blocked and reacted with the culture supernatants. Bound antibody was detected using an enzyme-labelled goat anti-mouse Ig antibody. Antibodies reacting specifically with p24 were selected (Figure 2). Designation of the strips is set forth in Table 2.

Table 2

Western Blot Analysis of Anti-p24 mAbs

Strip #	Designation
1	Positive Control
2	5-B4
3	5-D9
4	5-E2
5	5-F12
6	Positive Control
7	6-B9
8	6-C9
9	6-C10
10	6-E11
11	Positive Control
12	6-F6
13	10-B2
14	10-C12
15	Positive Control
16	10-D1
17	10-H1
18	7-D4
19	Positive Control
20	7-E1
21	7-E10
22	7-F3
23	8-E7
24	Positive Control
25	9-B7
26	9-D5

The anti-p24 antibodies were then tested for cross-reactivity to p26 of HIV-2 in immunoblots. HIV-2 lysates were separated, blotted and reacted with the anti-p24 antibodies. Two antibodies, 7-D4 and 5-D9 reacted strongly with p26 (Figure 3). Designation of the strips is set forth in Table 3.

Table 3

Cross-Reactivity of Anti-p24 mAbs with p26 of HIV-2

Strip	mAb
1	Hu-anti-HIV-1 IgG
2	OSS 39-B-3
3	MOPC 21 (IgG1)
4	F86/ 5-B4
5	5-D9
6	6-C10
7	7-E10
8	5-E2
9	9-B7
10	7-F3
11	6-F6
12	9-D5
13	7-D4
14	6-E11
15	7-E1

In a related experiment, 7-D4 recognized a protein of approximately 27,000 molecular weight in lysates of SIV_{MAC}.

Epitope Mapping

The amino acids that comprise the p24 epitope of 7-D4 were mapped in the following manner. The gag region and portions of gag were subcloned in an expression vector. Briefly, viral DNA of a λ_{BAT} bacteriophage (cDNA library HIV-1_{BF}, clone HAT 3 (Starcich et al., (1986)) was digested with EcoRI and by ligation into the pBR322-derived plasmid pMLB1113 to produce a plasmid identified as clone 29 which contained the EcoRI/SstI gag/pol ORF. Clone 29 was digested with SstI to remove extraneous vector sequences and religated to produce plasmid gag/pol 1.2. This latter plasmid was sonicated, blunt-ended and ligated with EcoRI linkers. The mixture was then digested with EcoRI, ligated into λORF8 (Meissner et al. 1987) and packaged. A λORF8 expression library was generated in E. coli and screened with a human anti-HIV polyclonal antibody and a mouse anti-p24 (HIV-1) monoclonal antibody. The positives were selected, expanded and the expressed peptides were characterized by Western blotting, immunoassay and nucleotide sequencing. The recombinant p24 peptides gag 8, gag 126, gag 107 and gag 141 were expressed in E. coli. Separately, clone 29 was used as a template and oligonucleotides corresponding to the 5' and 3' ends of the published sequence were used in a polymerase chain reaction to generate a complete sequence of the gag protein p24. The 5' end contained an EcoRI site and the 3' end contained a BamHI site. The reaction product was digested with EcoRI and BamHI and then ligated into pMLB1113. A recombinant p24 protein, gag 24.5, was expressed in E. coli. The characterization of the recombinant p24 peptides is presented in Figure 4.

The various recombinant p24 peptides were used as antigen in ELISA's and in Western blots to determine whether or not a given monoclonal antibody bound a given

peptide. The reactivity pattern of any one monoclonal antibody with the panel of p24 peptides allowed a localization of the recognized epitope to one of four regions as shown in Table 4 and Figures 5 and 6.

Table 4

Immunochemical Analysis of Anti-24 mAbs Using Recombinant Peptides

mAb	gag 24.5	gag 8	gag 126	gag 107	gag 141	mAb group
5-B4	+	-	+	-	-	B
5-D9	+	+	+	-	-	C
5-E2	+	+	-	-	-	D
5-F12	+	-	+	-	-	B
6-C10	+	+	+	-	-	C
6-E11	+	-	-	-	-	A
6-F6	+	-	-	-	-	A
7-D4	+	-	+	-	-	B
7-E1	+	-	+	-	-	B
7-E10	+	+	-	-	-	D
7-F3	+	-	+	-	-	B
8-E7	+	-	-	-	-	A
9-B7	+	+	-	-	-	D
9-D5	+	-	-	-	-	A
10-B2	+	-	+	-	-	B
10-C12	+	-	-	-	-	A

Because 7-D4 bound only to gag 24.5 and gag 126, it was possible to deduce that the 7-D4 epitope mapped to region B delimited by amino acid residues 142-209.

To further localize the epitope of 7-D4, synthetic sequential overlapping nonapeptides were made for the B region of p24. Each nonapeptide served as the solid phase antigen in a series of ELISA's to determine maximal binding affinity of the monoclonal. A single peak of reactivity was found (Figure 7) for a linear domain comprising the region containing amino acids 142-158 (Figure 8).

A comparison of the amino acid sequences of p24 of an HIV-1 isolate, p26 of an HIV-2 isolate and p27 of SIV_{MAC} revealed conservation of a decapeptide (Figure 9) within the epitope of p24 consisting of Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys. It can be inferred that the region encompassing the decapeptide is the 7-D4 epitope of p26 in HIV-2 and p27 in SIV_{MAC}.

The values of a defined epitope are known to those skilled in the art. One of the benefits is the ability of generating new antibodies capable of reacting with said epitope and similar epitopes. Synthetic peptides are configured after the epitope sequence and either unmodified or conjugated to carriers are used as antigen. For example, peptides can be conjugated to PPD, tetanus toxoid, KLH or BSA using glutaraldehyde, carbodiimide or N-maleimidobenzoyl hydroxuccinimide ester. For a review of using synthetic peptides as antigen, see Ciba Foundation Symposium 119 (1986) John Wiley and Sons, NY. Antibodies may be raised <u>in vivo</u> as in mice, goats or other lab animals or <u>in vitro</u> using a system of materials and methods similar to the IVIS of Hana Biologics (Alameda, CA). Another benefit is that large quantities of the epitope sequence can be produced synthetically or using standard recombinant DNA techniques as described above and the peptides can serve as antigen in immunology-based assays and kits for the detection of circulating antibody or for the detection of circulating antigen in an inhibition type assay.

Another benefit relates to improving the assays disclosed herein. Without extending the survey, it is unclear whether the epitope identified in the HIV-1 isolate described herein is specific to that isolate and furthermore to the HIV-2 and SIV isolates described herein. Using that sequence as a reference point, the epitope can be engineered, that is substituting one or more amino acids or alternatively derivitizing the epitope, etc., with a view to identifying a related sequence with a greater degree of conservation among a larger variety of HIV isolates or to obtaining a related sequence with a greater degree of reactivity in assays.

Capture ELISA Assay

To determine which of the monoclonals would find utility in an ELISA, each was used as a capture or HRP-conjugate antibody in a sandwich assay. Briefly, the monoclonal antibody was coated on wells and 10µl of disruption buffer added. The antigen samples suspended in detergent buffer or controls in a volume of 100µl were added next and incubated at 37°C for 90 minutes. After washing, bound antigen was detected by adding to the wells an enzyme conjugated anti-HIV reagent (horseradish peroxidase-conjugated human anti-HIV IgG, affinity purified, 100µl) and incubated at 37°C for 30 minutes. After washing several times, 100µl of substrate solution were added to the wells and incubated

at room temperature for 30 minutes. 100 µl of stop reagent were added and absorbance read at 450 nm using an air blank. Representative data are presented in Table 5.

Table 5

Checkerboard Analysis of mAbs

Capture Antibody	5B4	5D9	5E2	6C10	6E11	7E10	9B7	HαHIV
5B4	0.12	0.26	0.29	0.82	0.13	1.03	0.17	2.67
5D9	0.73	0.13	0.43	0.62	0.37	0.38	0.12	>3.0
5E2	0.58	0.47	0.14	0.61	0.23	0.80	0.11	2.51
6C10	0.81	0.38	0.44	0.20	0.17	0.70	0.13	>3.0
6E11	0.09	0.21	0.21	0.14	0.16	0.27	0.09	0.41
7E10	0.84	0.43	0.49	0.84	0.18	0.18	0.13	>3.0
9B7	0.14	0.11	0.10	0.17	0.13	0.17	0.13	0.28
34A	0.49	0.12	0.08	0.96	0.28	1.81	0.22	>3.0

Purified mAb were coated overnight at 10 µg/ml. HRP-mAb used at 10 µg/ml added at beginning of incubation (90′ at 37°C).

HRP-human-anti-HIV was added after 60 min.

Absorbances given for 10.0 ng/ml HIV-1 MOLT 3 in NHS.

Absorbance for NHS was 0.12 ± 0.03

Antibodies 5-B4, 6-C10 and 7-E10 worked best as both capture and conjugated antibodies. Maximal signals were obtained with the HRP-human anti-HIV as the conjugate.

Various combinations of the monoclonals were used as capture antibodies in ELISA's. The combination of 5-

B4 and 6-C10 showed the greatest sensitivity in detecting p24 (Figure 10). To detect p26 of HIV-2, 7-D4 was used as a capture antibody (Figure 11). It was found that maximal sensitivity and robustness occurred when the three antibodies, 5-B4, 6-C10 and 7-D4 were combined as capture antibodies. Under those conditions, p26 was detectable as well as p24 from certain borderline clinical samples that were difficult to interpret when only 5-B4 and 6-C10 were used as capture antibodies. The sensitivity of the capture ELISA using these three antibodies is less than 10 pg/ml (less than 1 pg/well) of HIV-1 p24 antigen and less than 0.5 ng/ml of HIV-2 p26 antigen (Figure 12). The sensitivity is found despite the presence of HIV antibodies in the clinical samples. A capture ELISA using the three antibodies 5-B4, 6-C10 and 7-D4 was also compared to a reverse transcriptase assay for the detection of whole virus. The ELISA was 25,000 times more sensitive than the reverse transcriptase assay (Figure 13).

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that this disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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The claims defining the invention are as follows:-

1. A monoclonal antibody which reacts with an epitope of p24 of HIV-1 and p26 of HIV-2, said epitope located within amino acid residues 140-160 of p24.

2. The monoclonal antibody of claim 1 wherein said epitope is located within amino acid residues 142-158 of p24.

3. The monoclonal antibody of claim 1 wherein said epitope is located within amino acid residues 144-158 of p24.

4. The monoclonal antibody of claim 1 wherein said epitope comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

5. The monoclonal antibody of claim 1 wherein said epitope comprises the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

6. A monoclonal antibody which reacts with an antigen consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

7. A monoclonal antibody which reacts with an antigen consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

8. An epitope consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function and with which the monoclonal antibody of claim 1 reacts.

9. An epitope consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys with which the monoclonal antibody of claim 1 reacts.

10. An amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

11. An amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

12. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which reacts with an antigen of HIV-1 and a monoclonal antibody of claim 1.

13. The diagnostic kit of claim 12 wherein said antibody is a monoclonal antibody.

14. The diagnostic kit of claim 13 wherein the epitope of said antibody comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

15. The diagnostic kit of claim 13 wherein one of said monoclonal antibodies which react with an antigen of HIV-1 binds with an epitope located within amino acid residues 142-209 of p24 and the second of said monoclonal antibodies which react with an antigen of HIV-1 binds with an epitope located within amino acid residues 263-344 of p24.

16. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which react with an antigen of HIV-1 and the monoclonal antibody of claim 6.

17. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which react with an antigen of HIV-1 and the monoclonal antibody of claim 7.

18. A method for detection of HIV-1 and HIV-2 antigens in a sample which

comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 1, and measuring the formation of antigen-antibody complexes.

19. The method of claim 18 wherein said antibody is a monoclonal antibody.

20. The method of claim 19 wherein the epitope of said antibody comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

21. The method of claim 19 wherein one of said monoclonal antibodies which react with an antigen of HIV-1 binds with an epitope located within amino acid residues 142-209 of p24 and the second of said monoclonal antibodies which react with an antigen of HIV-1 binds with an epitope located within amino acid residues 263-344 of p24.

22. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 6, and measuring the formation of antigen-antibody complexes.

23. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 7, and measuring the formation of antigen-antibody complexes.

24. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 8 and measuring the formation of antigen-antibody complexes.

25. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 9 and measuring the formation of antigen-antibody complexes.

26. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the amino acid sequence of claim 10 and measuring the formation of antigen-antibody complexes.

27. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the amino acid sequence of claim 11 and measuring the formation of antigen-antibody complexes.

28. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample consisting of the epitope of claim 8.

29. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample consisting of the epitope of claim 9.

30. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the amino acid sequence of claim 10.

31. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the amino acid sequence of claim 11.

32. A monoclonal antibody which reacts with an epitope of p24 of HIV-1 and p26

of HIV-2, said epitope located within amino acid residues 140-160 of p24, substantially as herein described with reference to the Example but excluding any comparative examples.

33. A monoclonal antibody which reacts with an antigen consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function, substantially as herein described with reference to the Example but excluding any comparative examples.

34. A monoclonal antibody which reacts with an antigen consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys, substantially as herein described with reference to the Example but excluding any comparative examples.

35. An epitope consisting of the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function and with which the monoclonal antibody of claim 32 reacts.

36. An epitope consisting of the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys with which the monoclonal antibody of claim 32 reacts.

37. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which reacts with an antigen of HIV-1 and a monoclonal antibody of any one of claims 32 to 34.

38. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of any one of claims 32 to 34, and measuring the formation of antigen-antibody complexes.

39. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 35 or 36 and measuring the formation of antigen-antibody complexes.

40. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample consisting of the epitope of claim 35 or 36.

Dated 6 August, 1993
Akzo N.V.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

FIG. 1

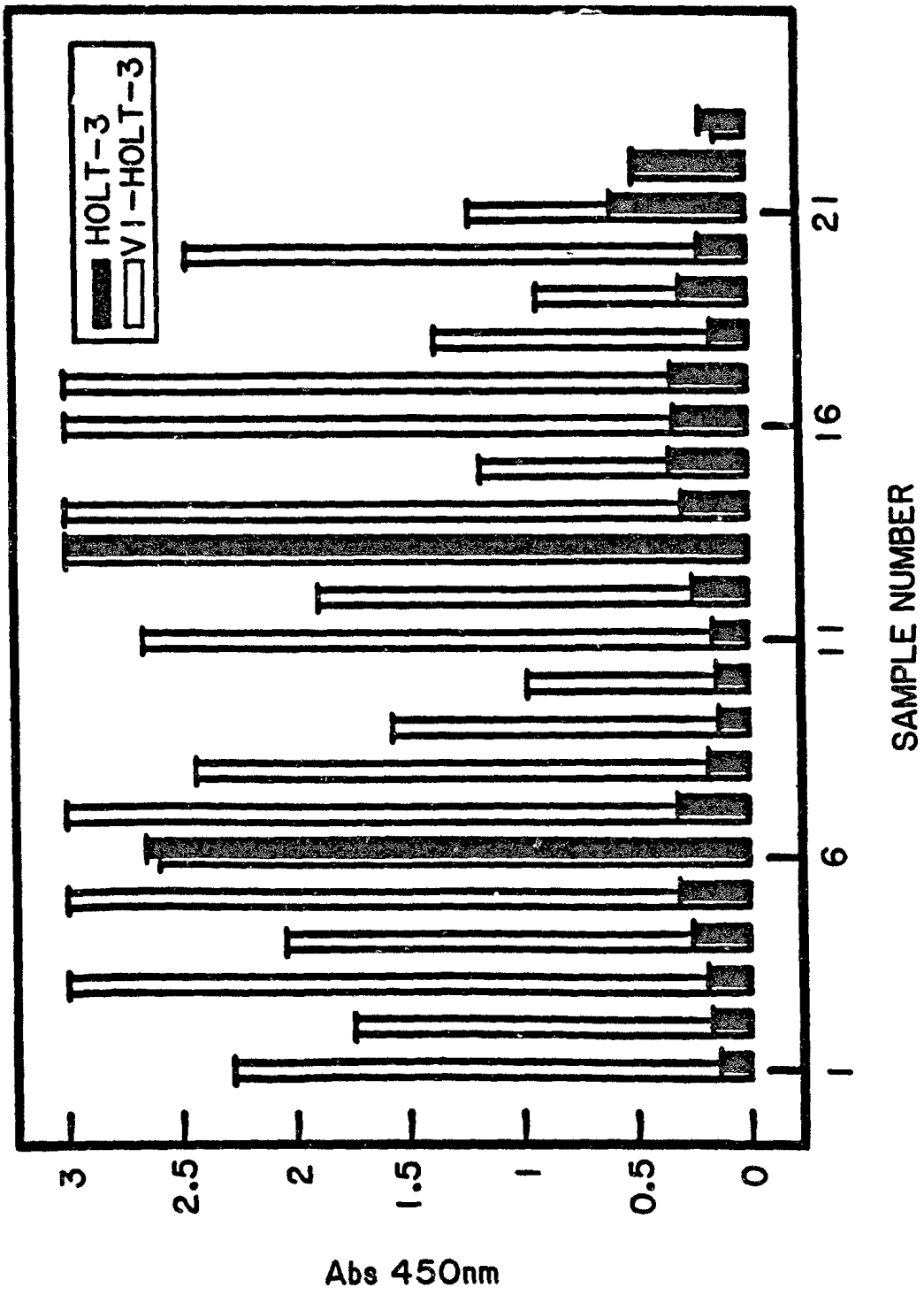

HOLT-3
VI-HOLT-3
3
2.5
2
1.5
1
0.5
0
Abs 450nm
1
6
11
16
21
SAMPLE NUMBER

FIG. 2a

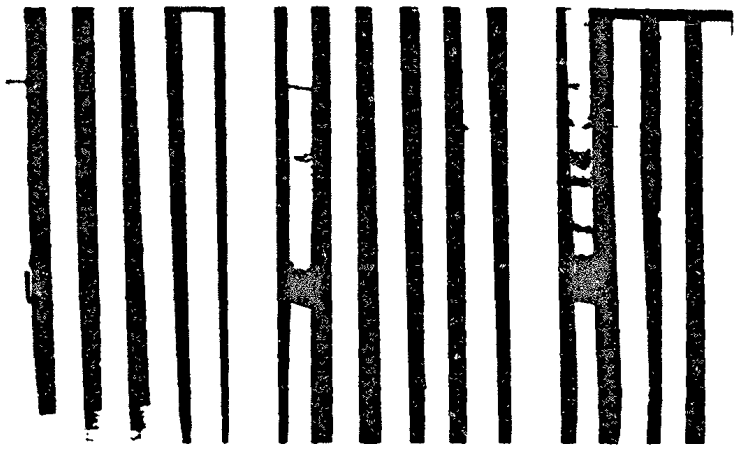


FIG. 2b

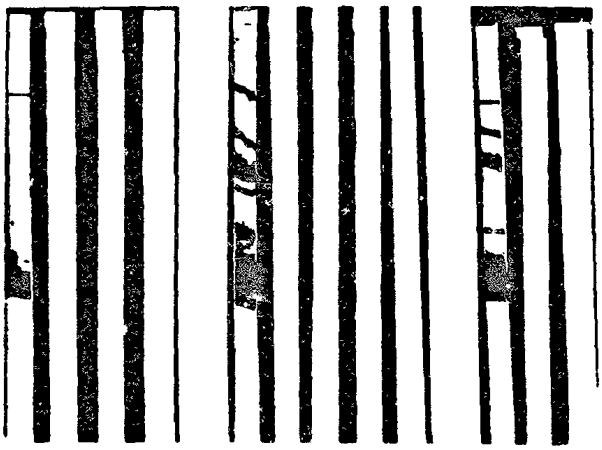
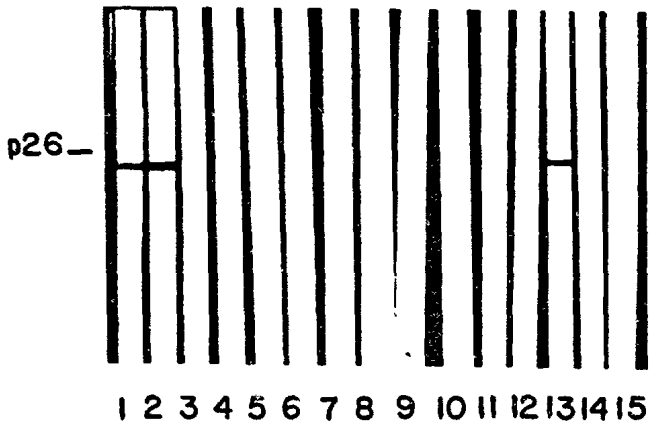


FIG. 3



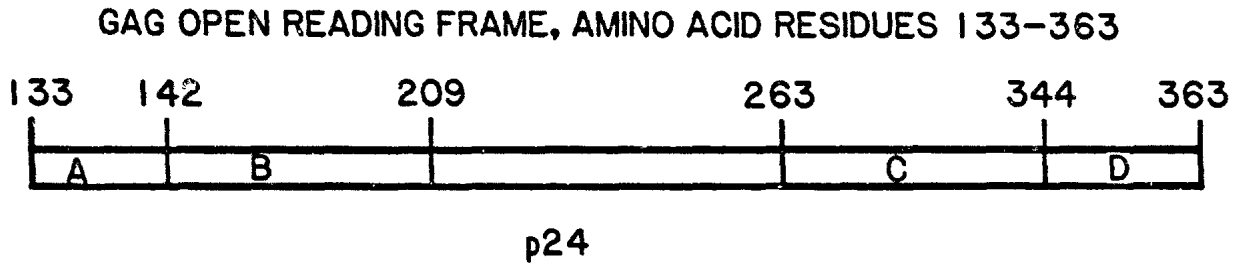

GAG OPEN READING FRAME, AMINO ACID RESIDUES 133-363
133
142
209
263
344
363
A
B
C
D
p24

FIG. 5

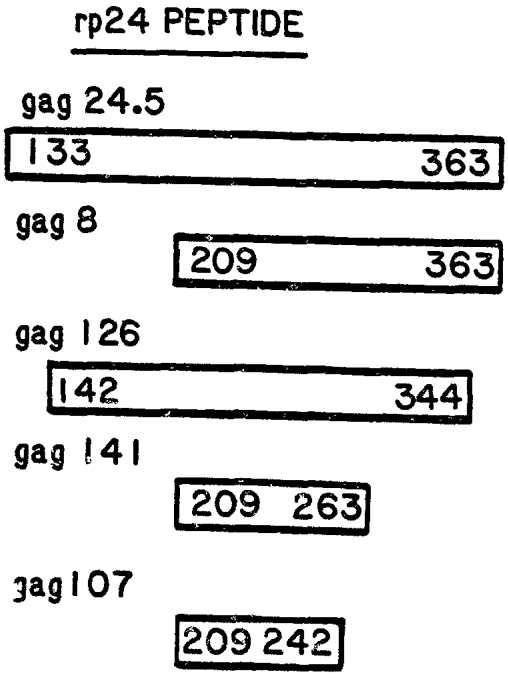

rp24 PEPTIDE
gag 24.5
133
363
gag 8
209
363
gag 126
142
344
gag 141
209 263
gag107
209 242

FIG. 4

FIG. 6a

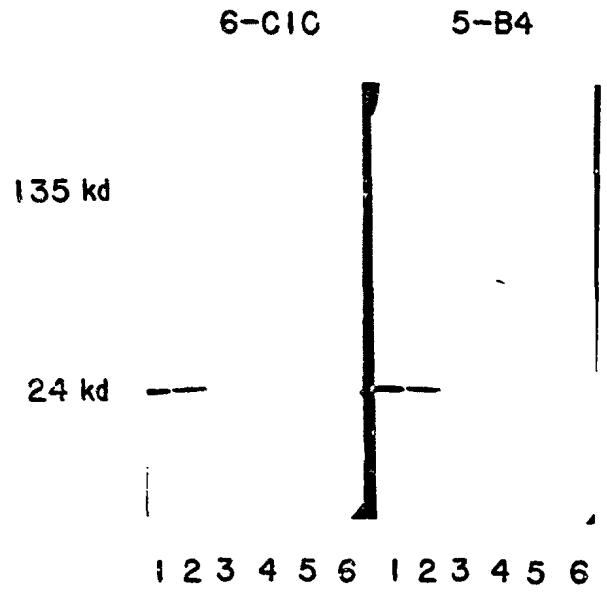

6-C1C
5-B4
135 kd
24 kd
1 2 3 4 5 6 1 2 3 4 5 6

FIG. 6b


7-D4
7-E10
135 kd
24 kd
1 2 3 4 5 6 1 2 3 4 5 6

FIG. 7

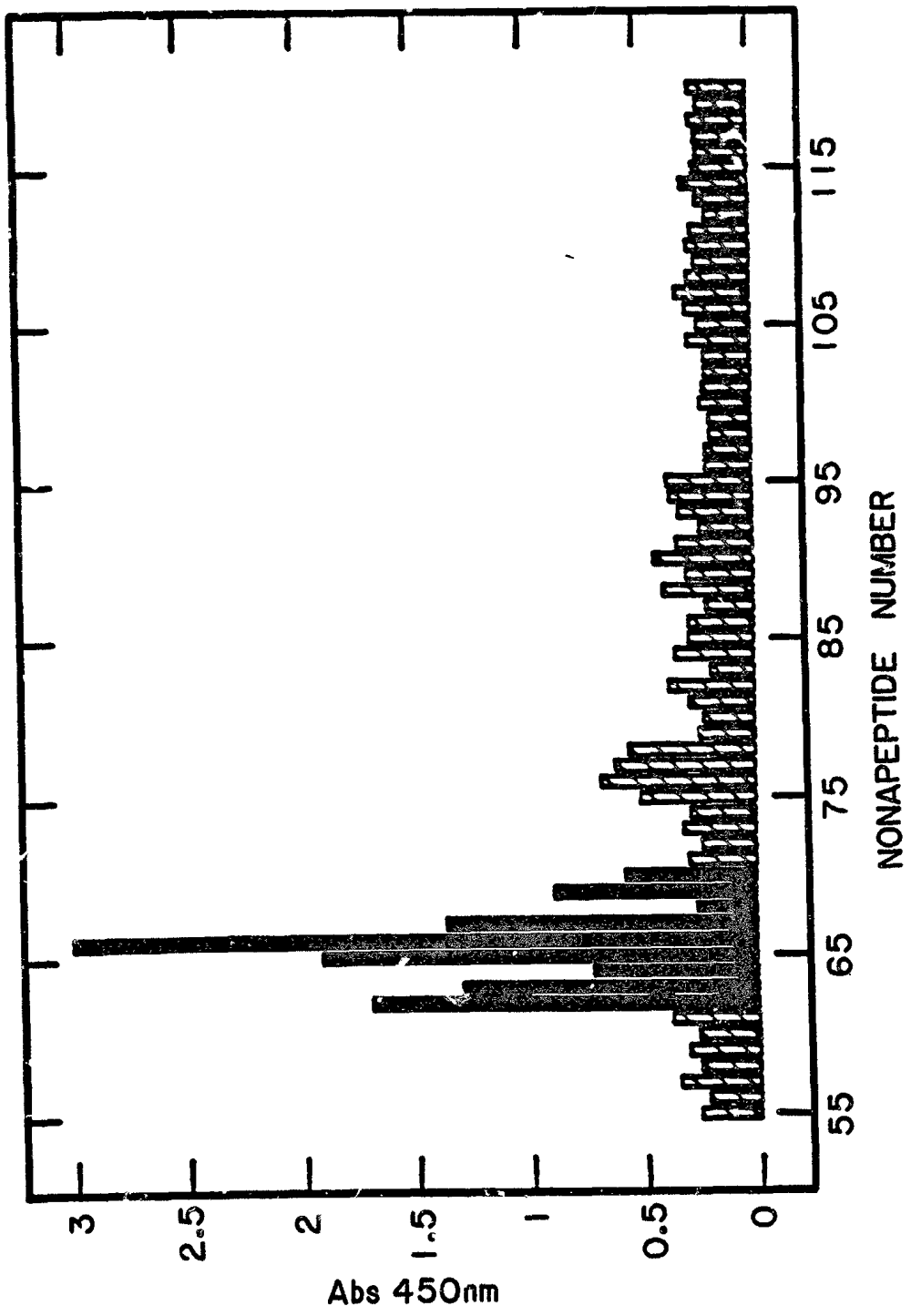

NONAPEPTIDE NUMBER
55
65
75
85
95
105
115
Abs 450nm
0
0.5
1
1.5
2
2.5
3

FIG. 8

NONAPEPTIDE FRACTION	AMINO ACID SEQUENCE	ELISA
61	Gln-Met-Val-His-Gln-Ala-Ile-Ser-Pro	-
62	Met-Val-His-Gln-Ala-Ile-Ser-Pro-Arg	+
63	Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr	+
64	His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu	+
65	Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn	+
66	Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala	+
67	Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp	+
68	Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val	-
69	Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys	+
70	Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-Val	+
71	Thr-Leu-Asn-Ala-Trp-Val-Lys-Val-Val	-
COMPOSITE (amino acid residues 142-158):		
$HIV\text{-}1_{RF}$:	-Gln-Met-Val-[His]-Gln-Ala-Ile-[Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys]-Val-[Val] (142, 158)	+

FIG. 9

	142														158		
HIV-1_{RF}:	-Gln-	Met-	Val-	His-	Gln-	Ala-	Ile-	Ser	-Pro-	Arg-	Thr-	Leu-	Asn-	Ala-	Trp-	Val-	Lys-	Val-	Val-	+
HIV-2_{NIH-Z}:	-Asn-	Tyr-	Thr-	His-	Ile-	Pro-	Leu-	Ser-	Pro-	Arg-	Thr-	Leu-	Asn-	Ala-	Trp-	Val-	Lys-	Leu-	Val-	+
SIV_{MAC}:	-Asn-	Tyr-	Thr-	His-	Leu-	Pro-	Leu-	Ser-	Pro-	Arg-	Thr-	Leu-	Asn-	Ala-	Trp-	Val-	Lys-	Leu-	Val-	+

FIG. 10

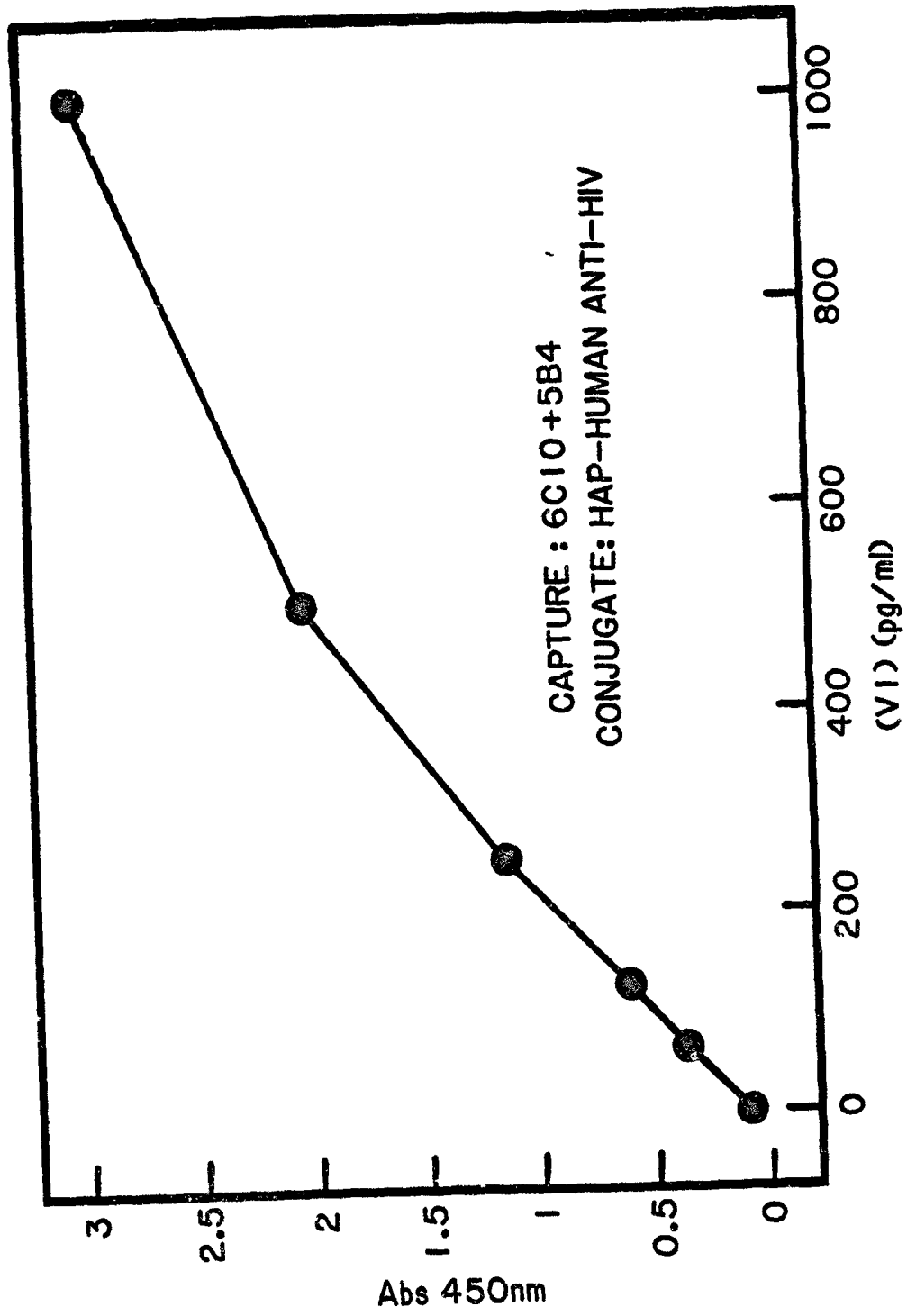

CAPTURE : 6C10+5B4
CONJUGATE: HAP-HUMAN ANTI-HIV
Abs 450nm
3
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
(V1) (pg/ml)

FIG. 11

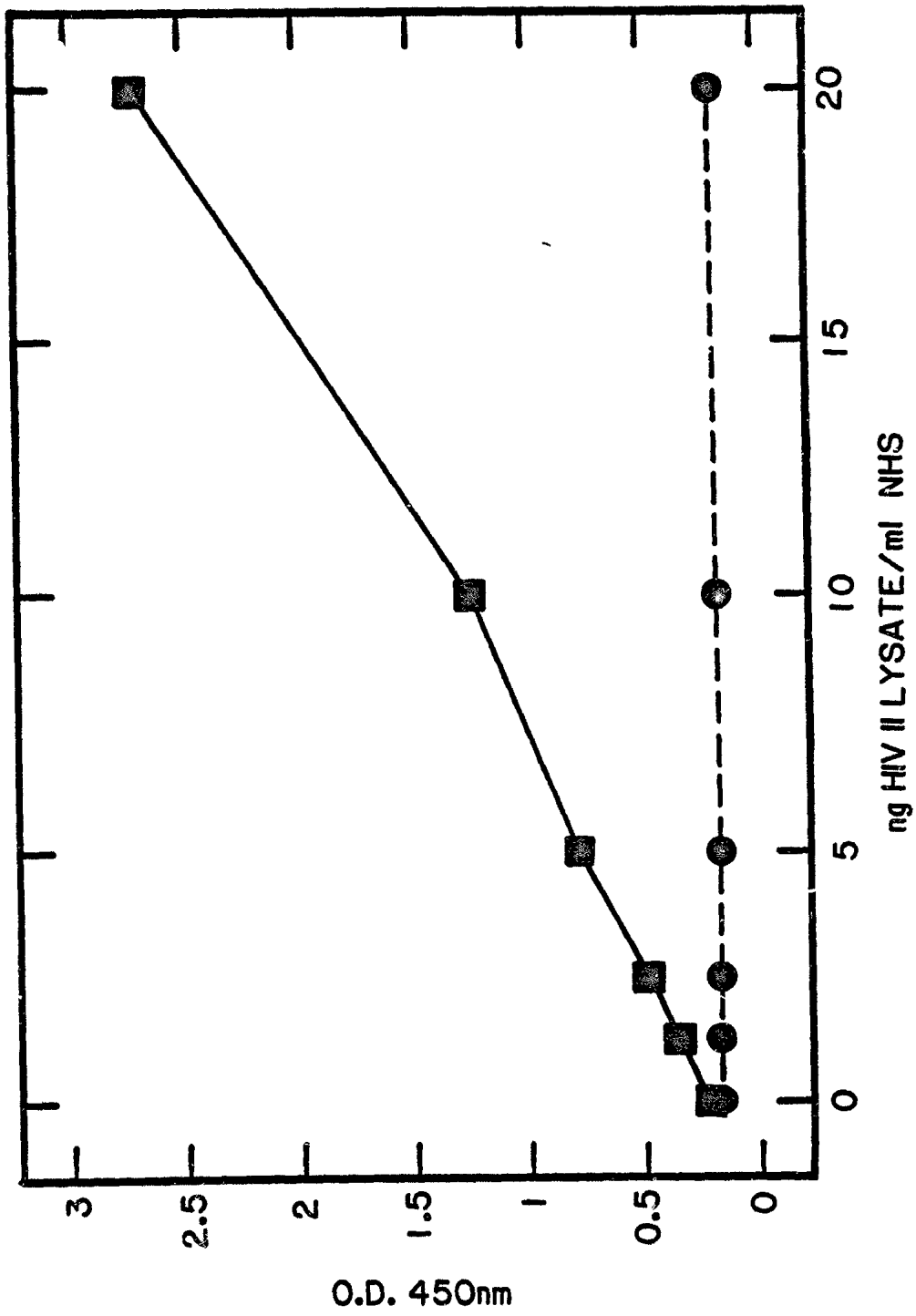
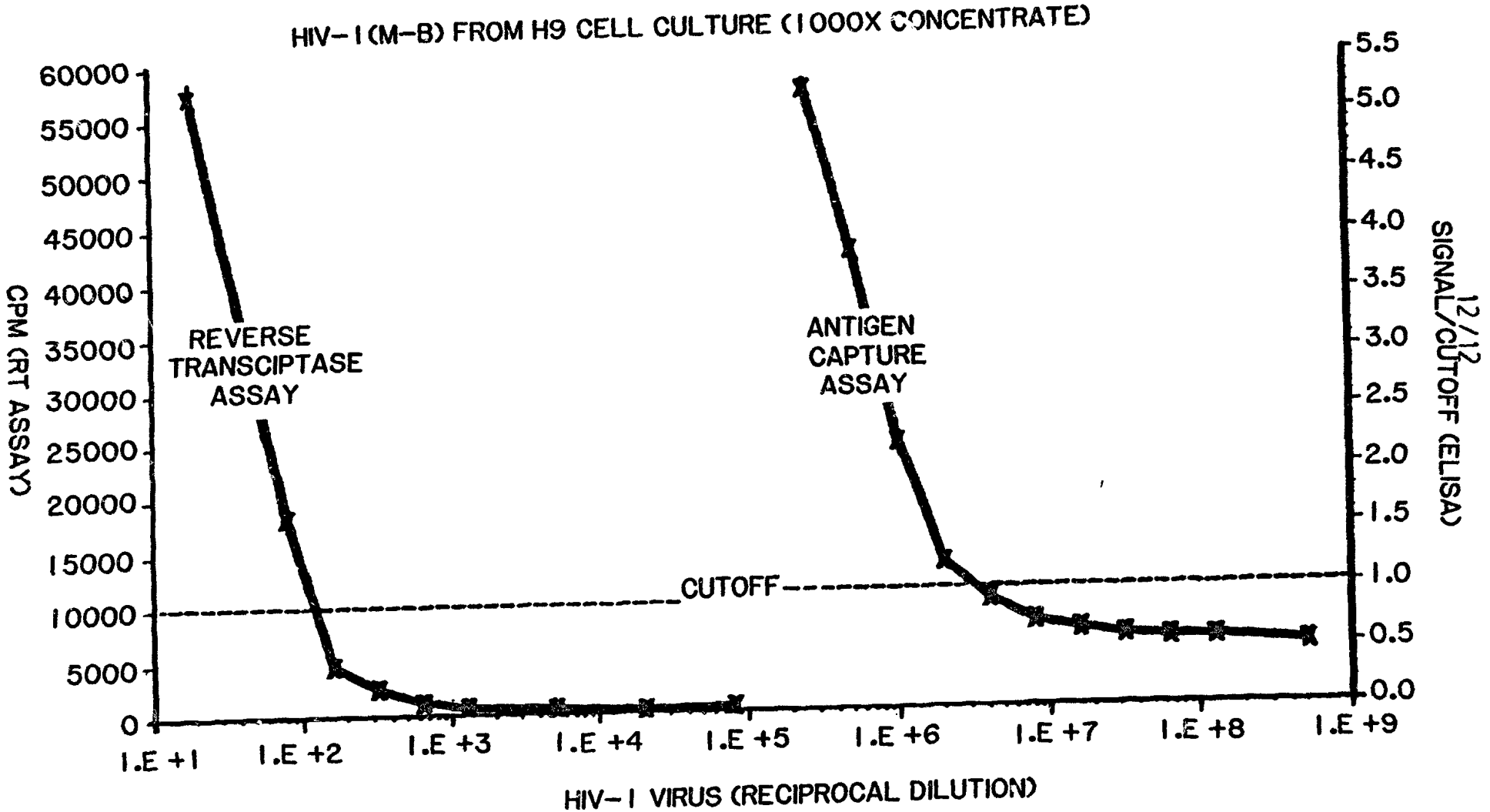


FIG. 12

PCT/US90/02874

FIG. 13


HIV-1 (M-B) FROM H9 CELL CULTURE (1000X CONCENTRATE)
CPM (RT ASSAY)
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
REVERSE TRANSCIPTASE ASSAY
ANTIGEN CAPTURE ASSAY
CUTOFF
1.E +1
1.E +2
1.E +3
1.E +4
1.E +5
1.E +6
1.E +7
1.E +8
1.E +9
HIV-1 VIRUS (RECIPROCAL DILUTION)
SIGNAL/CUTOFF (ELISA)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02874

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07K 7/06,7/08,15/28; G01N 33/543,569,571,577

U.S.Cl.: 435/5, 7, 810; 436/570, 578,548; 530/327,328,387,806,809; 935/110

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/5,7,810; 436/510,518,548; 530/327,328,387, 806,809; 935/110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

BIOSIS searches: 1) Monoclonal (10A) (ARV OR HIV or LAV or HIV) and (P24 or P26);
2) (PEPTIDE # or OLIGOPEPTIDE #) and (ARV or HTLV or LAV

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
P,X	US, A, 4,843,011 (SARNGADHARAN et al.) 27 June 1989. See col. 5, lines 35-56 2nd col. 6, lines 38-60.	1-7
P,X	US, A, 4,888,290 (KORTRIGHT et al.) 19 December 1989. See col. 2, lines 55-64 and col. 4, line 42-col. 5, line 5.	1-7, 12, 17-19, 24 and 25
X	Biological Abstracts, vol. 86, No. 10, issued 15 November 1988, M. Niedrig et al., "Monoclonal antibodies directed against human immunodeficiency virus (HIV) gag proteins with specificity for conserved epitopes in HIV-2 and simian immunodeficiency virus". See page 1270, col. 1, the abstract no. 109338.	1-7
P,X,	Biological Abstracts, vol. 87, no. 10, issued 15 May 1989, P, Kusk et al., "Immunological characterization and detection of the major core protein p24 of the human immunodeficiency virus (HIV) using monoclonal antibodies". See page 1146, col. 1, the abstract no. 110752.	1-7, and 12-25.

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [1]	Date of Mailing of this International Search Report [1]
15 August 1990	04SEP 1990
International Searching Authority [1]	**Signature of Authorized Officer [2]**
ISA/US	NGUYEN NGOC-HO INTERNATIONAL DIVISION David A. Saunders

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Biological Abstracts, Vol. 84, no. 1, issued 01 July 1987, T. Hattori et al., "Characteracterization of three monoclonal antibodies (VAK3-5) that identify p24, core protein of human immunodeficiency virus and its precursors". See page 499, col. 1, the abstract no. 445.9	1-7
X	Biological Abstracts, vol. 80, no. 10, issued 15 November 1985, V. DI Marzo et al., "Monoclonal antibodies specific for p24, the major core protein of human T cell leukemia virus type III". See page 587, col. 2, the abstract no. 87756.	1-7

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
X	US, A, 4,755,457 ROBERT-GUROFF et al.) 05 July 1988. See col. 2, lines 27-29.	1-7

continued from I. Classification of subject matter block 3.

or HIV) and (P24 or P26).