

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 200210043 B2
(10) Patent No. 785387

(54) Title
Sequences and methods for detection of HIV-1

(51) International Patent Classification(s)
C12Q 1/70 (2006.01) **C12N 15/09** (2006.01)
C12Q 1/68 (2006.01)

(21) Application No: **200210043**

(22) Application Date: **2002.01.04**

(30) Priority Data

(31) Number	(32) Date	(33) Country
09757207	2001.01.09	US

(43) Publication Date : **2002.07.11**

(43) Publication Journal Date : **2002.07.11**

(44) Accepted Journal Date : **2007.03.29**

(71) Applicant(s)
Becton, Dickson and Company

(72) Inventor(s)
Tobin J Hellyer; James M Harris; Qimin You

(74) Agent/Attorney
F B Rice and Co,Level 23,44 Market Street,SYDNEY NSW 2000

(56) Related Art
DATABASE GENBANK, 18 DEC 2000, ACC. NO. AJ4059548
DATABASE EMBL, 9 JUNE 2000, ACC. NO. AF221023

ABSTRACT

Primers and probes derived from the HIV-1 *pol* gene which facilitate detection and/or quantification of all presently known genotypes of HIV-1 (A-I and O). Disclosed sequences may be
5 used in a variety of amplification and non-amplification formats for detection of HIV nucleic acids.



AUSTRALIA

Patents Act 1990



Becton, Dickinson and Company

COMPLETE SPECIFICATION STANDARD PATENT

Invention Title:

Sequences and methods for detection of HIV-1

The following statement is a full description of this invention including the best method of performing it known to us:-

FIELD OF THE INVENTION

10 The present invention relates to materials and methods for detection of HIV nucleic acids, in particular to probes and primers for detection of HIV in hybridization and amplification assays.

BACKGROUND OF THE INVENTION

15 The genome of the Human Immunodeficiency Virus (HIV) is highly heterogeneous and exhibits a mutation rate on the order of 10^{-4} per base per generation. Combined with the rapid rate of viral propagation in infected individuals, this presents particular challenges for diagnostic nucleic acid amplification techniques which typically amplify a single conserved sequence within a target organism. Although a great deal of research activity has been directed to detecting HIV-1 in
20 hybridization and amplification assays, such molecular assays have so far been limited by their inability to detect all genotypes with equal efficiency. Although a signal amplification system for the detection of HIV-1 type O has been reported, none of the currently available diagnostic nucleic acid amplification methods are able to detect this genotype of the virus.

25 SUMMARY OF THE INVENTION

 The present invention provides primers and probes derived from the HIV-1 *pol* gene which facilitate detection and/or quantification of all presently known genotypes of HIV-1 (A-I and O). A single amplification primer pair according to the invention efficiently amplifies all known genotypes of
30 HIV-1, which may then be detected in a single detection step using the detector probes and primers of the invention.

DESCRIPTION OF THE DRAWINGS

35 Fig. 1 shows the titration of internal control fluorescent signal (FAM) with increasing levels of native HIV target (ROX).

DETAILED DESCRIPTION OF THE INVENTION

The primers, hybridization probes and detector probes of the present invention are complementary to regions of the HIV-1 polymerase (*pol*) gene. Initially, design of the disclosed primers and probes was based on conserved regions in an alignment of fifty-eight HIV-1 *pol* sequences. Additional data was subsequently added to the alignment to provide a database with a total of 115 *pol* sequences. One goal was to develop probes and primers which, in spite of the heterogeneity of the *pol* sequence, provided amplification, detection and/or quantitation of all presently known HIV-1 genotypes with essentially equal efficiency in amplification reactions. In some cases this was accomplished by overlapping the hybridization site of the 5' ends of certain of the detector probes with the hybridization site of the 3' end an amplification primer. This approach took advantage of sequence conservation in the SDA primer region and avoided much of the sequence heterogeneity evident in the intervening region between the two SDA primers. This technique also allowed use of a smaller target sequence, thereby improving amplification efficiency.

As used herein, an amplification primer is an oligonucleotide for amplification of a target sequence by extension of the oligonucleotide after hybridization to the target sequence or by ligation of multiple oligonucleotides which are adjacent when hybridized to the target sequence. At least a portion of the amplification primer hybridizes to the target. This portion is referred to as the target binding sequence and it determines the target-specificity of the primer. In addition to the target binding sequence, certain amplification methods require specialized non-target binding sequences in the amplification primer. These specialized sequences are necessary for the amplification reaction to proceed and typically serve to append the specialized sequence to the target. For example, the amplification primers used in SDA include a restriction endonuclease recognition site 5' to the target binding sequence (US Patent Nos. 5,270,184 and 5,455,166). NASBA, 3SR and transcription based amplification primers require an RNA polymerase promoter linked to the target binding sequence of the primer. Linking such specialized sequences to a target binding sequence for use in a selected amplification reaction is routine in the art. In contrast, amplification methods such as PCR, which do not require specialized sequences at the ends of the target, generally employ amplification primers consisting of only target binding sequence.

As used herein, the terms "primer" and "probe" refer to the function of the oligonucleotide. A primer is typically extended by polymerase or ligation following hybridization to the target but a probe typically is not. A hybridized oligonucleotide may function as a probe if it is used to capture or detect a target sequence, and the same oligonucleotide may function as a primer when it is employed as a target binding sequence in an amplification primer. It will therefore be appreciated that any of the target binding sequences disclosed herein for amplification, detection or quantitation of HIV-1 may be used either as hybridization probes or as target binding sequences in primers for detection or amplification, optionally linked to a specialized sequence required by the selected amplification reaction or to facilitate detection.

Based on the alignment of multiple HIV-1 *pol*/gene sequences, the following amplification primers were designed for testing in SDA reactions. Target binding sequences are underlined. The remaining 5' portion of the sequence comprises the restriction endonuclease recognition site (RERS) that is required for the SDA reaction to proceed and a generic non-target-specific tail sequence. It will be readily apparent that the target binding sequences may be used alone to amplify the target in reactions which do not require specialized sequences or structures (e.g., PCR) and that different specialized sequences required by amplification reactions other than SDA may be substituted for the RERS-containing sequence shown below (e.g., an RNA polymerase promoter). "R" and "L" in the primer name indicates "right" and "left" primers, respectively, when the oligonucleotides are used in amplification reactions:

AMPLIFICATION PRIMERS

QAL48	CGATTCCGCTCCAGACTTCTCGGGTAGATACAGGAGCAGAT	SEQ ID NO:1
QAL46	CGATTCCGCTCCAGACTTCTCGGGAGATACAGGAGCAGAT	SEQ ID NO:2
QAR48	ACCGCATCGAATGCATGTCTCGGGCTATCATTTTGGTTTCC	SEQ ID NO:3
QAR44	ACCGCATCGAATGCATGTCTCGGGTATCATTTTGGTTTCC	SEQ ID NO:4
AL46	CGATTCCGCTCCAGACTTCTCGGGCAGTACAAATGGCAGT	SEQ ID NO:5
AL48	CGATTCCGCTCCAGACTTCTCGGGGAGTACAAATGGCAG	SEQ ID NO:6
AL50	CGATTCCGCTCCAGACTTCTCGGGGAGTACAAATGGCAGT	SEQ ID NO:7
AR44	ACCGCATCGAATGACTGTCTCGGGTGTACCCCCCAATC	SEQ ID NO:8
AR44B	ACCGCATCGAATGACTGTCTCGGGCTGTACCCCCCAAT	SEQ ID NO:9
AR48	ACCGCATCGAATGACTGTCTCGGGTGTACCCCCCAATCC	SEQ ID NO:10

In addition, the following detector primers were designed for real-time detection of amplification products produced using the amplification primers. The structure and use of such detector primers is described, for example, in US Patent No. 5,547,861 and US Patent No. 5,928,869. The target binding sequences are underlined. The remaining portion of the sequence forms a hairpin structure which is typically labeled to facilitate detection of amplification products as is known in the art. It will be readily apparent that the target sequence may be used alone for direct detection by hybridization (typically linked to a detectable label) and that other directly and indirectly detectable sequences and labels may be substituted for the hairpin as is known in the art. See, for example US Patent No. 5,935,791; US Patent No. 5,846,726; US Patent No. 5,691,145; US Patent No. 5,550,025 and US Patent No. 5,593,867.

DETECTOR PRIMERS

QDR66	TAGACCCGAGTGCTGGCAAATTCATTTCTTCTAATACTG	SEQ ID NO:11
QDR64	TAGACCCGAGTGCTGCAAATTCATTTCTTCTAATACTGT	SEQ ID NO:12
QDR56	TAGACCCGAGTGCTAAATTCATTTCTTCTAATACTGT	SEQ ID NO:13

QOL1	TAGCACCCGAGTGCTAGGAGCAGATGATACAGT	SEQ ID NO:14
QOL2	TAGCACCCGAGTGCTCAGGAGCAGATGATACAGT	SEQ ID NO:15
QOL3	TAGCACCCGAGTGCTACAGGAGCAGATGATACAGT	SEQ ID NO:16
QOL4	TAGCACCCGAGTGCTGAGCAGATGATACAGT	SEQ ID NO:17
QOL5	TAGCACCCGAGTGCTGGAGCAGATGATACAGT	SEQ ID NO:18
DL56HPD	TAGCACCCGAGTGCTCACAATGTTAAAAGAAAAGGG	SEQ ID NO:19
DL52HPD	TAGCACCCGAGTGCTACAATGTTAAAAGAAAAGGG	SEQ ID NO:20
DL50HPD	TAGCACCCGAGTGCTCAATGTTAAAAGAAAAGGG	SEQ ID NO:21
DR58HPD	TAGCACCCGAGTGCTCCCTTTTCTATTAAAATTGTG	SEQ ID NO:22
DR54HPD	TAGCACCCGAGTGCTCCCTTTTCTATTAAAATTGTG	SEQ ID NO:23
DR52HPD	TAGCACCCGAGTGCTCCCTTTTCTATTAAAATTG	SEQ ID NO:24
DR48HPD	TAGCACCCGAGTGCTCCCTTTTCTATTAAAATTG	SEQ ID NO:25
Pol2DR58	TAGCACCCGAGTGCTCCCTTTTCTTTTAAAATTGTG	SEQ ID NO:26
DN2	TAGCACCCGAGTGCTCCCAATCCCCCTTTTCTGTTAAAAT	SEQ ID NO:27
DN3	TAGCACCCGAGTGCTCCCAATCCCCCTTTTCTGTTAAAAT	SEQ ID NO:28
DN4	TAGCACCCGAGTGCTCCAATCCCCCTTTTCTGTTAAAAT	SEQ ID NO:29
DN5	TAGCACCCGAGTGCTCAATCCCCCTTTTCTGTTAAAAT	SEQ ID NO:30
DN5.1	TAGCACCCGAGTGCTCAATCCCCCTTTTCTTTTAAAAT	SEQ ID NO:31
OL62	TAGCACCCGAGTGCTCCCAATCCCCCTTTTCTTTT	SEQ ID NO:32
OL64	TAGCACCCGAGTGCTCCCAATCCCCCTTTTCTTTTA	SEQ ID NO:33

SEQ ID NOs:11-13 and 19-26 are conventional non-overlapping detector primers which contain a hairpin as described in US Patent No. 5,928,869. SEQ ID NOs:14-18 and 27-33 also contain the hairpin but the 5' end of the target binding sequences overlap with the 3' end of the target binding sequences of the upstream amplification primers.

Bumper primers used in SDA (BR and BL) were also designed. The entire sequence of these oligonucleotides consists of target binding sequence:

BUMPER PRIMERS/DETECTOR PROBES

QBL44	CTAAAGGAAGCTCTAT	SEQ ID NO:34
QBR42	AACCTCCAATTCCC	SEQ ID NO:35
BL54	GAATCTATGAATAAAGAATTAAA	SEQ ID NO:36
BR54	TGCTATTATGTCTACTATTCT	SEQ ID NO:37

The sequences set forth above were selected to minimize the effects of heterogeneity in the targeted region of the *pol* gene. Mismatches were confined to the middle or the 5' end of the sequences to permit efficient 3' extension upon hybridization to the target sequence. Only three of the 117 HIV-1 strains analyzed exhibit more than one mismatch with amplification primers SEQ ID

NO:5 and SEQ ID NO:9 or with detector SEQ ID NO:26. Detector SEQ ID NO:27-30 contain a deliberate mismatch eight bases from the 3' end to minimize target-specific variations in priming efficiency caused by heterogeneity in the target region.

Because the target binding sequence confers target specificity on the primer or probe, it should be understood that the target binding sequences exemplified above for use as particular components of a specific amplification reaction may also be used in a variety of other ways for detection of HIV. For example, the target binding sequences of SEQ ID NOs:1-37 may alternatively be used as hybridization probes for direct detection of HIV-1, with or without prior amplification or as a post-amplification assay. Such hybridization methods are well known in the art and typically employ a detectable label associated with or linked to the target binding sequence to facilitate detection of hybridization. Further, essentially all of the target binding sequences set forth above may be used as amplification primers in amplification reactions which do not require additional specialized sequences (such as PCR) or appended to the appropriate specialized sequences for use in 3SR, NASBA, transcription-based or any other primer extension amplification reactions. For detection of amplification products, amplification primers comprising the target binding sequences disclosed herein may be labelled as is known in the art, or labelled detector primers comprising the disclosed target binding sequences may be used in conjunction with the amplification primers as described in US Patent No. 5,547,861 and US Patent No. 5,928,869 for real-time homogeneous detection of amplification. Such detector primers typically comprise a directly or indirectly detectable sequence which does not initially hybridize to the target but which facilitates detection of the detector primer once it has hybridized to the target and been extended. For example, such detectable sequences may be sequences which form a secondary structure, sequences which contain a restriction site, or linear sequences which are detected by hybridization of their complements to a labelled oligonucleotide (sometimes referred to as a reporter probe) as is known in the art. Alternatively, the amplification products may be detected post-amplification by hybridization of a probe selected from any of the target binding sequences disclosed herein which fall between a selected set of amplification primers.

It is to be understood that an oligonucleotide according to the invention which consists of a target binding sequence and, optionally, either a sequence required for a selected amplification reaction or a sequence required for a selected detection reaction may also include certain other sequences which serve as spacers, linkers, sequences for labelling or binding of an enzyme, etc. Such additional sequences are typically known to be necessary to obtain optimum function of the oligonucleotide in the selected reaction and are intended to be included by the term "consisting of".

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

EXAMPLE 1

SDA reactions were performed to determine the analytical sensitivity of the assay for the detection of HIV DNA. Amplification was carried in the presence of 0, 5, 10, 50, 100 or 250 copies of

HIV target DNA. The target sequence was a fragment of the HIV-1 genome corresponding to nucleotides 4659-4910 of strain B-WEAU (GenBank accession number U21135) that was cloned into the plasmid vector pBlueScript SK+ (Stratagene). SDA was performed at 52°C using 500nM primers (SEQ ID NO:5 and SEQ ID NO:9), 50nM bumpers (SEQ ID NO:36 and SEQ ID NO:37), and 200nM detector probe, SEQ ID NO:26, in buffer containing: 100mM bicine; 30mM potassium hydroxide; 68mM K_iPO₄, pH7.6; 10.5% glycerol; 6.5% DMSO; 0.7mM dC₅TP; 0.1mM dA-, dG- and dTTP; 70ng/μl human placental DNA; 100ng/μl bovine serum albumin; 4mM magnesium acetate; 6U *Bst* polymerase and 32U *Bso*BI restriction enzyme. The detector primer was labeled at the 5' terminus with a fluorescein donor molecule and internally with a dabcyI quencher moiety. The two dyes on the detector were separated by a *Bso*BI restriction endonuclease recognition sequence and held in close juxtaposition by a hairpin structure within the tail sequence as described in US Patent Nos. 5,919,630; 5,928,869 and 5,958,700. Donor fluorescence was monitored during the course of amplification.

In the presence of target, donor fluorescence increased during the course of the reaction as the hairpin holding the donor and quencher in close proximity unfolded and the restriction site was cleaved. In contrast, in the absence of target, fluorescence remained consistently low throughout the reaction. Results were expressed in terms of area under the curve or "MOTA." A larger MOTA score indicates generation of more fluorescence and, generally, the presence of more input target. The results are shown in Table 1.

TABLE 1

TARGETS PER REACTION	MEAN MOTA SCORE (n=28)*
0	606
5	14883
10	20565
50	47643
100	56740
150	61676

* n=54 for negative controls

The limit of detection (LOD), defined as the input target level at which 95% of reactions would yield a positive result, was determined to be between 9 and 11 copies of target DNA depending on the MOTA score selected as a cut-off for determining a positive result. These results demonstrate that the amplification and detector primers are capable of sensitive and reproducible detection of HIV DNA.

EXAMPLE 2

A similar experiment to that described in Example 1 was performed using the SEQ ID NO:27 detector primer, the target hybridization region of which overlaps the SEQ ID NO:9 amplification primer. This probe was designed to take advantage of sequence conservation in the amplification primer binding region while maintaining the specificity afforded by detection of the internal region of the SDA amplicon that lies between the two amplification primers. Buffer conditions were the same as described above with the following modifications: 75mM K₂PO₄, 14% DMSO, 5% glycerol and 27U *Bst*BI. The limit of detection using the overlapping probe was 61 to 86 copies of HIV target DNA depending on the MOTA score selected for determining a positive result. These results demonstrate sensitive and specific detection of HIV target DNA using an alternative probe design that offers additional flexibility in the development of SDA-based systems.

EXAMPLE 3

The SDA assay for HIV DNA was converted to a two-step reverse transcriptase (RT)-SDA format in which RNA was first copied to cDNA using an RT enzyme and then amplified in a conventional SDA reaction. One important difference between the SDA conditions described in Examples 1 and 2 and the RT-SDA described here is the absence of bumper primers. The need for bumpers apparently was precluded by the choice of an RT enzyme which possesses RNase H activity and which is therefore capable of degrading the RNA template following reverse transcription, thereby liberating a single stranded DNA target sequence in to solution. An LOD study was performed for HIV RNA using *in vitro* transcripts of a genotype B strain generated from the pBlueScript plasmid clone described in Example 1.

Reverse transcription was carried out in microtiter wells using 15U avian myeloblastosis virus (AMV)-RT in buffer containing: 69.3mM bicine; 12.3mM KOH; 20.8mM K₂PO₄; 3% glycerol; 4.5% DMSO; 6mM magnesium acetate; 2.75mM dC₃TP; 0.25mM dA-, dG- and dTTP; 100ng/μl BSA; 1250nM SEQ ID NO:5 and 700nM SEQ ID NO:9. In brief, the reverse transcription reaction mixture without enzyme was incubated for 10 min at 68°C to denature the target RNA. Eighty microliters of denatured target was then added to a microwell containing 20μl of an RT enzyme mixture that was pre-equilibrated at 50°C. The complete RT reaction was incubated for 10 min to facilitate synthesis of cDNA and degradation of the RNA template by the RNase H activity of the RT enzyme. To initiate amplification, 40μl of the reverse transcription reaction was transferred to a second microtiter well at 52°C containing 60μl of an amplification mixture comprising *Bst* polymerase, *Bst*BI restriction enzyme, and fluorescent detector primer together with certain buffer components. The final SDA conditions were as follows: 72mM bicine; 24mM potassium hydroxide; 54.4mM K₂PO₄; 4% glycerol; 11% DMSO; 0.1mM dA-, dG- and dTTP; 1.1mM dC₃TP; 700ng/μl hpDNA; 100ng/μl BSA; 500nM SEQ ID NO:5; 300nM SEQ ID NO:9; 600nM SEQ ID NO:26; 12U *Bst*BI and 7U *Bst* polymerase. The wells

were sealed and incubated at 52°C. Donor fluorescence was monitored throughout the course of the reaction.

The results of this experiment are shown in Table 2.

5

RNA TARGETS PER REACTION	MEAN MOTA SCORE (n=17)	% POSITIVE
0	139	0
10	3577	64.7
25	7810	88.0
50	13410	100.0
75	23641	100.0
100	35719	100.0
500	73870	100.0

MOTA scores ≥ 1000 were considered positive. All reactions containing ≥ 50 copies of HIV target RNA were positive as were 88% containing 25 copies and 65% of those containing 10 copies. These results demonstrate the sensitivity of the disclosed amplification and detector primers for the detection of HIV-1 RNA.

10

EXAMPLE 4

RT-SDA was performed on purified RNA from representative isolates of nine different clades of HIV-1 (Boston Biomedica, Inc.). In order to quantify the parental stocks of viral RNA, all except the type O nucleic acid were tested with each of two commercially available quantitative HIV tests: the Roche Amplicor HIV-1 Monitor V1.5 and the Chiron Quantiplex HIV-1 RNA 3.0 Assay. Since neither of these tests is able to amplify RNA of HIV-1 genotype O, a third non-amplified system, the Digene Hybrid Capture Assay, was used to quantify the type O nucleic acid. Viral RNAs for genotypes A-H were diluted according to the results of the Roche assay to give the equivalent of 200 copies per RT-SDA reaction. Type O RNA was diluted to the same level based on the results of the Digene test. RT-SDA was performed according to the method described in Example 3. For eight of the nine clades, all sixteen replicates were positive at 200 copies per RT-SDA reaction. For genotype F, 4/16 (25%) replicates were positive. The reason for the apparently lower sensitivity with type F is unclear but might be attributed to inaccurate quantification of the parental RNA stock of this genotype by the Roche assay. Alternatively, the discrepancy might be due to the presence in the type F sequence of a single base mismatch with the right amplification primer which is used to initiate first strand cDNA synthesis (SEQ ID NO:9). Importantly, all reactions conducted with 2000 input copies of type F RNA were positive.

15

20

25

These data demonstrate that the disclosed primers and probes are capable of detecting multiple genotypes of HIV-1, including type O, with a high degree of sensitivity. Detection of type O RNA is particularly important in view of the inability to detect this clade with the majority of commercially available diagnostic nucleic acid assays.

5

EXAMPLE 5

In vitro transcripts generated from the plasmid clone described in Example 1 were quantified by competitive RT-SDA. In brief, RT-SDA was performed as described above with the exception that two detector primers were included in the reaction mixture, both at a concentration of 200nM. The first primer, SEQ ID NO:26, was specific for HIV-1 and was labeled at the 5' end with dabcy1 quencher moiety and internally with rhodamine (Rox). The second probe was specific for an internal control sequence and was labeled at its 5' end with fluorescein and internally with a dabcy1 quencher. The internal control comprised an RNA molecule that was generated by *in vitro* transcription of a mutated clone of the HIV-1 *pol* gene. The internal control possessed the same primer binding regions as the native HIV target but differed by a series of point mutations introduced to coincide with the 3' end of the detector hybridization region. These mutations permitted discrimination of the native target and internal control by preventing hybridization and extension of the mismatched probes during amplification. The internal control was designed such that it amplified with similar efficiency to the native target and would compete during the course of the reaction for one or more rate-limiting reagents. The same amount of internal control was seeded into each test sample and into a series of calibrator reactions containing known amounts of native target. Details of the theoretical aspects of the quantitative SDA algorithm may be found elsewhere (J. G. Nadeau, et al. 1999. *Anal Biochem* **276**, 177-187; C. M. Nycz, et al. 1998. *Anal Biochem* **259**, 226-234). In brief, the fluorescence produced by amplification of both the native target and internal control was monitored at discreet intervals throughout the reaction. Data were processed through a series of normalization and smoothing functions to produce a fluorescent signal for both sequences. From these values, the natural log of the ratio of native target and internal control signals was calculated. The values obtained from the calibrator wells were plotted as a regression against the natural log of the input number of target molecules of RNA to produce calibration curves corresponding to each time point. An automated algorithm was then used to determine the time at which it was statistically optimal to perform quantification.

Fig. 1 shows the MOTA scores for the internal control with various copy numbers of input target sequence. MOTA scores for the internal control decreased with increasing levels of native target, demonstrating the competition between the two targets that is necessary for accurate quantification. Table 3 shows the results of quantitative competitive RT-SDA with between 50 and 100000 copies of native RNA target and 5000 copies of internal control per reaction. At all but one

35

target level, accuracy and precision were better than $\pm 25\%$, thus demonstrating the ability to quantify HIV-1 RNA using the disclosed amplification and detector primer sequences.

TABLE 3

TARGET RNA COPIES/REACTION		MEAN (n=8)	PERCENT	
REVERSE TRANSCR.	SDA*		ACCURACY	PRECISION
0	0	16		62.78
125	50	47	-5.17	25.47
1250	500	537	7.45	24.19
12500	5000	8765	75.29	9.07
125000	50000	60009	20.02	5.48
250000	100000	76351	-23.65	7.26

5 * Based on 100% conversion of RNA to cDNA and amplification of 40% of the reverse transcription in the SDA reaction

Sequence alignment data and initial testing of the additional target binding sequences disclosed herein indicates that similar results would be obtained using these sequences in probes and primers for amplification and/or detection of all genotypes of HIV-1.

Page(s) 18-20 are claims pages
They appear after the sequence listing(s)

SEQUENCE LISTING

<110> Hellyer, Tobin J.
 You, Qimin
 5 Harris, James M.

 <120> Sequences and Methods for Detection of HIV-1

 <130> Seq/Mtds for Detection of HIV-1
 10
 <140>
 <141>

 <160> 37
 15
 <170> PatentIn Ver. 2.1

 <210> 1
 <211> 41
 20 <212> DNA
 <213> Human immunodeficiency virus type 1

 <400> 1
 25 cgattccgct ccagacttct cgggtagata caggagcaga t 41

 <210> 2
 <211> 40
 <212> DNA
 30 <213> Human immunodeficiency virus type 1

 <400> 2
 cgattccgct ccagacttct cgggagatac aggagcagat 40
 35
 <210> 3
 <211> 42
 <212> DNA
 <213> Human immunodeficiency virus type 1
 40
 <400> 3
 accgcacga atgcatgtct cgggctatca ttttggttt cc 42

 45 <210> 4
 <211> 41
 <212> DNA
 <213> Human immunodeficiency virus type 1

 50 <400> 4
 accgcacga atgcatgtct cgggtatcat ttttggtttc c 41

 <210> 5
 55 <211> 40
 <212> DNA
 <213> Human immunodeficiency virus type 1

 <400> 5
 60 cgattccgct ccagacttct cgggcagtac aaatggcagt 40

<210> 6
 <211> 40
 <212> DNA
 5 <213> Human immunodeficiency virus type 1

 <400> 6
 cgattccgct ccagacttct cggggcagta caaatggcag 40

 10
 <210> 7
 <211> 41
 <212> DNA
 <213> Human immunodeficiency virus type 1
 15
 <400> 7
 cgattccgct ccagacttct cggggcagta caaatggcag t 41

 20
 <210> 8
 <211> 38
 <212> DNA
 <213> Human immunodeficiency virus type 1
 25
 <400> 8
 accgcatcga atgactgtct cgggtgtacc ccccaatc 38

 30
 <210> 9
 <211> 38
 <212> DNA
 <213> Human immunodeficiency virus type 1
 35
 <400> 9
 accgcatcga atgactgtct cgggtgtac cccccaat 38

 40
 <210> 10
 <211> 39
 <212> DNA
 <213> Human immunodeficiency virus type 1
 45
 <400> 10
 accgcatcga atgactgtct cgggtgtacc ccccaatcc 39

 50
 <210> 11
 <211> 40
 <212> DNA
 <213> Human immunodeficiency virus type 1
 <400> 11
 tagcaccgga gtgctggcaa attcatttct tctaatactg 40

 55
 <210> 12
 <211> 40
 <212> DNA
 <213> Human immunodeficiency virus type 1
 60
 <400> 12

tagcacccga gtgctgcaaa ttcatttctt ctaatactgt

40

<210> 13

5 <211> 38

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 13

10 tagcacccga gtgctaaatt catttcttct aatactgt

38

<210> 14

<211> 38

15 <212> DNA

<213> Human immunodeficiency virus type 1

<400> 14

20 tagcacccga gtgctaggag cagatgatacagt

38

<210> 15

<211> 34

<212> DNA

25 <213> Human immunodeficiency virus type 1

<400> 15

tagcacccga gtgctcagga gcagatgata cagt

34

<210> 16

<211> 35

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 16

tagcacccga gtgctacagg agcagatgat acagt

35

<210> 17

<211> 31

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 17

tagcacccga gtgctgagca gatgatacag t

31

<210> 18

50 <211> 32

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 18

55 tagcacccga gtgctggagc agatgataca gt

32

<210> 19

<211> 36

60 <212> DNA

<213> Human immunodeficiency virus type 1

<400> 19
 tagcacccga gtgctcaciaa tgttaaaaga aaaggg 36

5
 <210> 20
 <211> 35
 <212> DNA
 <213> Human immunodeficiency virus type 1

10
 <400> 20
 tagcacccga gtgctacaat gttaaaagaa aaggg 35

15
 <210> 21
 <211> 34
 <212> DNA
 <213> Human immunodeficiency virus type 1

20
 <400> 21
 tagcacccga gtgctcaatg ttataaagaaa aggg 34

25
 <210> 22
 <211> 37
 <212> DNA
 <213> Human immunodeficiency virus type 1

30
 <400> 22
 tagcacccga gtgctcccct tttctattaa aattgtg 37

35
 <210> 23
 <211> 36
 <212> DNA
 <213> Human immunodeficiency virus type 1

40
 <400> 23
 tagcacccga gtgctccctt ttctattaaa attgtg 36

45
 <210> 24
 <211> 35
 <212> DNA
 <213> Human immunodeficiency virus type 1

<400> 24
 tagcacccga gtgctcccct tttctattaa aattg 35

50
 <210> 25
 <211> 34
 <212> DNA
 <213> Human immunodeficiency virus type 1

55
 <400> 25
 tagcacccga gtgctccctt ttctattaaa attg 34

60
 <210> 26
 <211> 37

<212> DNA
<213> Human immunodeficiency virus type 1

<400> 26

5 tagcacccga gtgctcccct tttcttttaa aattgtg 37

<210> 27

<211> 41

10 <212> DNA

<213> Human immunodeficiency virus type 1

<400> 27

15 tagcacccga gtgctcccaa tcccccttt tctgttaaaa t 41

<210> 28

<211> 42

<212> DNA

20 <213> Human immunodeficiency virus type 1

<400> 28

tagcacccga gtgctcccaa atccccctt tctgttaaaa at 42

<210> 29

<211> 40

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 29

tagcacccga gtgctccaat ccccccttt ctgttaaaat 40

<210> 30

<211> 39

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 30

tagcacccga gtgctcaatc ccccttttc tggttaaaat 39

<210> 31

<211> 39

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 31

50 tagcacccga gtgctcaatc ccccttttc ttttaaaat 39

<210> 32

<211> 36

55 <212> DNA

<213> Human immunodeficiency virus type 1

<400> 32

60 tagcacccga gtgctcccaa tcccccttt tctttt 36

<210> 33
<211> 37
<212> DNA
<213> Human immunodeficiency virus type 1

5

<400> 33
tagcacccga gtgctcccaa tcccccttt tctttta 37

10 <210> 34
<211> 16
<212> DNA
<213> Human immunodeficiency virus type 1

15 <400> 34
ctaaaggaag ctctat 16

20 <210> 35
<211> 14
<212> DNA
<213> Human immunodeficiency virus type 1

25 <400> 35
aacctccaat tccc 14

30 <210> 36
<211> 23
<212> DNA
<213> Human immunodeficiency virus type 1

35 <400> 36
gaatctatga ataaagaatt aaa 23

40 <210> 37
<211> 21
<212> DNA
<213> Human immunodeficiency virus type 1

45 <400> 37
tgctattatg tctactattc t 21

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method for detecting an HIV-1 target sequence comprising:
 - (a) amplifying the target sequence using a first amplification primer having a sequence consisting of the target binding sequence of any one of SEQ ID NO:1 or SEQ ID NO:3 and, optionally, a sequence required for a selected amplification reaction, and;
 - (b) detecting the amplified target sequence.
2. The method of claim 1 further comprising a second amplification primer having a sequence consisting of the target binding sequence of any one of SEQ ID NO:1 or SEQ ID NO:3 and, optionally a sequence required for a selected amplification reaction.
3. The method of claim 1 wherein the target binding sequence of the first amplification primer is the target binding sequence of SEQ ID NO:1.
4. The method of claim 2 wherein the target binding sequence of the second amplification primer is the target binding sequence of SEQ ID NO:3.
5. The method of claim 1 further comprising quantifying the target sequence.
6. The method of claim 5 wherein the target sequence is quantified by co amplification of a control sequence and the target sequence.
7. The method of claim 1 wherein multiple HIV-1 genotypes are detected.
8. An oligonucleotide having a sequence consisting of the target binding sequence of any one of SEQ ID NO:1 or SEQ ID NO:3 and, optionally, either a sequence required for a selected amplification reaction or a sequence required for a selected detection reaction.
9. The oligonucleotide of claim 8 which consists of the target binding sequence of SEQ ID NO:1 and, optionally, a sequence required for a selected amplification reaction.
10. The oligonucleotide of claim 8 which consists of the target binding sequence of SEQ ID NO:3 and, optionally, a sequence required for a selection detection reaction.

20

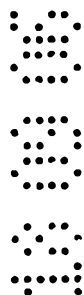
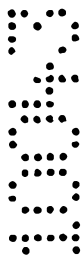
11. The oligonucleotide of claim 10 wherein the sequence required for the detection reaction is a hairpin, a G-quartet, a restriction site or a sequence which hybridizes to a reporter probe.

Dated this fourteenth day of September 2006

Becton, Dickinson and Company
Patent Attorneys for the Applicant:

F B RICE & CO

5



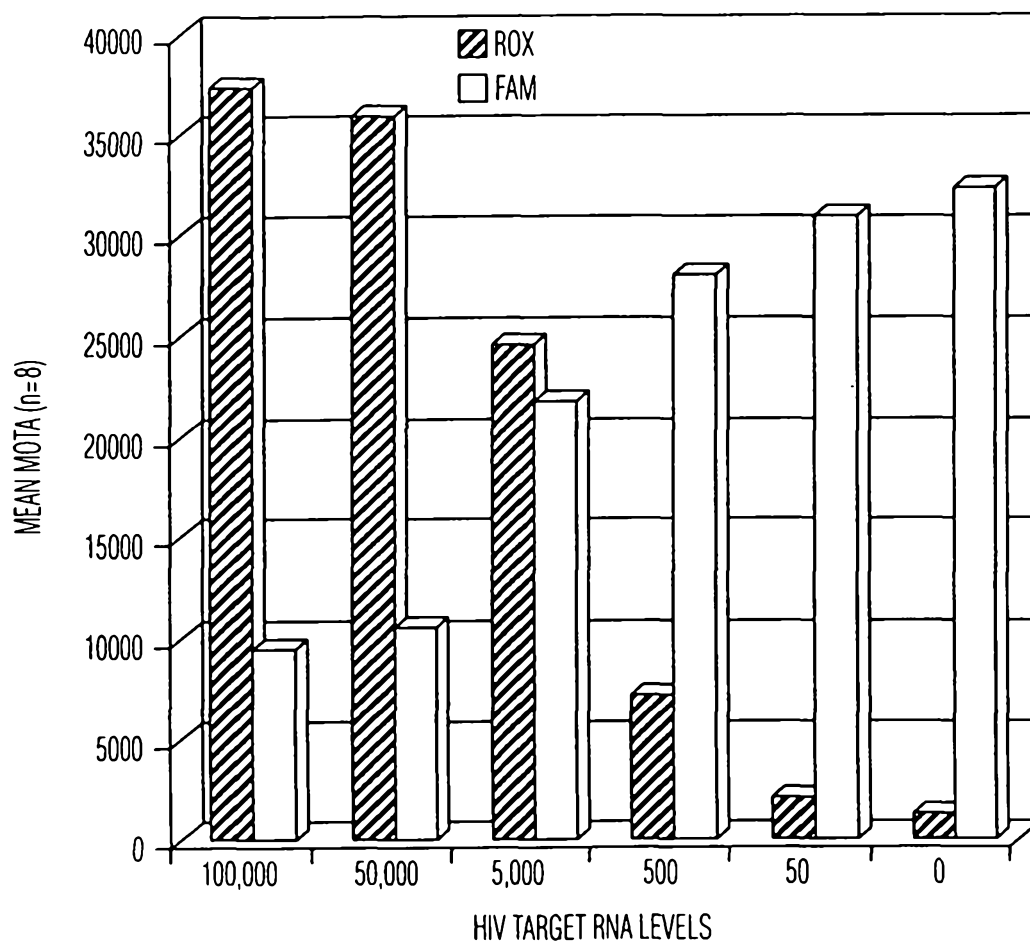


FIG. 1