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Comparative Evaluation of Three Assays for the Quantitation of Human Immunodeficiency Virus Type 1 RNA in Plasma

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Reverse transcriptase-coupled polymerase chain reaction (Amplicor HIV-1 Monitor), the branched DNA (bDNA) method (Quantiplex HIV-1 RNA) and the nucleic acid sequence-based assay (NASBA HIV-1 RNA QT) were comparatively evaluated for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma. Among 60 plasma specimens from HIV-1 infected patients, HIV-1 RNA was detected in 56 by Amplicor (sensitivity, 93.3%), in 41 by bDNA (sensitivity, 68.3%), and in 60 by NASBA (sensitivity, 100%). HIV-1 RNA was not detected by any of these methods in 34/34 plasma specimens from HIV-1-seronegative blood donors (specificity, 100%). The HIV-1 RNA levels as determined by the different methods were correlated significantly. The frequency of concordant results (log difference <0.50) was 80.4% between Amplicor and NASBA, 77.5% between Amplicor and bDNA, and 58.6% between bDNA and NASBA. After initiation of antiviral therapy, HIV-1 RNA level variations observed with the three methods were similar. HIV-1 RNA levels were inversely correlated with the CD4+ T cell counts, whereas no correlation was found with HIV-1 p24-antigen levels. When the methods were evaluated for reproducibility, coefficients of variation ranged from 11% to 40% for Amplicor, from 6% to 35% for bDNA, and from 13% to 62% for NASBA. Quantitation of HIV-1 RNA in culture supernatants from HIV-1 subtype A to H strains showed that bDNA can be used to quantitate RNA from all HIV-1 subtypes, whereas Amplicor failed to detect RNA from subtype A strains and NASBA subtype G strains.

KEY WORDS: HIV-1, viral load, amplicor, bDNA, NASBA

INTRODUCTION

Quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma represents a useful tool for the management of patients with HIV-1 infection. Indeed, HIV-1 RNA can be detected during all stages of HIV-1 infection [Piatak et al., 1993]. During the asymptomatic period, the HIV-1 RNA level in plasma remains stable, reflecting an equilibrium between HIV-1 replication rate and efficacy of immune response [Henrard et al., 1995]. In contrast, an increase in the HIV-1 RNA plasma level is correlated with disease progression [Holodniy et al., 1991; Semple et al., 1991; van Kerckhoven et al., 1994; Verhofstede et al., 1994]. Studies carried out on patients with long-term non-progressive HIV-1 infection have shown that very low HIV-1 RNA levels are found in these patients, compared with patients with progressive HIV-1 disease [Cao et al., 1995; Pantaleo et al., 1995]. It has also been shown that early in HIV-1 infection, the HIV-1 RNA level in plasma is predictive of the clinical course of the disease [Hogervorst et al., 1995] and that plasma levels of HIV-1 RNA predict clinical outcome independently of CD4+ lymphocyte counts [Mellors et al., 1995, 1996]. Taken together, these data indicate that the HIV-1 RNA level in plasma could represent a more relevant criteria than the CD4+ lymphocyte count for the initiation of antiretroviral therapy.

On the other hand, it has been shown that in patients treated with anti-retroviral drugs, the efficacy of treatment seems to be correlated with a dramatic decrease in the HIV-1 RNA level in plasma, whereas either loss of drug activity or treatment discontinuation are associ-

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ated with an increase in RNA level [Piatak et al., 1993; Schuurman et al., 1995; Loveday et al., 1995; Semple et al., 1991; O'Brien et al., 1996]. Furthermore, compared with other virological markers such as HIV-1-p24 antigenemia or HIV-1 proviral DNA burden in peripheral blood cells, the HIV-1 RNA level in plasma has been found to be the only marker which reflects the downregulation of viral replication in lymphoid tissue during the course of antiviral therapy [Cohen et al., 1995]. Therefore, HIV-1 RNA level in plasma can now be considered as the most suitable marker to predict disease progression and to assess the efficacy of antiretroviral therapies.

Until recently, quantitative reverse transcription-coupled polymerase chain reaction (RT-PCR) was used to determine the HIV-1 RNA level in plasma. However, in-house quantitative RT-PCR procedures, which are exceedingly labor intensive, are not convenient for large-scale use. They also suffer from laboratory-to-laboratory variation due to the lack of standardization [Lin et al., 1994].

HIV-1 RNA quantitation in plasma can now be carried out by using commercial assays. The Amplicor HIV-1 Monitor assay (Roche Diagnostics Systems, Neuilly, France) is based on the RT-PCR technology. The quantitation procedure relies on co-amplification of an internal RNA standard; amplified products are detected thereafter by a nonradioactive detection system [Mulder et al., 1994]. The Quantiplex HIV RNA assay (Chiron Diagnostics, Cergy-Pontoise, France) is based on branched DNA (bDNA) signal amplification technology. The direct detection of HIV-1 RNA involves a series of nucleic acid hybridizations. The captured HIV-1 RNA target sequences are hybridized with multiple branched DNA molecules that serves as amplifier. After hybridization of enzyme-labeled probes with the amplifier, the RNA-probe complex is detected by use of chemiluminescent substrate [Dewar et al., 1994; Pachl et al., 1994]. The HIV-1 RNA QT assay (Organon Teknika, Fresnes, France) involves the nucleic acid sequence-based amplification (NASBA) procedure, which is based on isothermal amplification of an HIV-1 RNA target sequence by the simultaneous enzymatic activity of avian myelobastosis virus reverse transcriptase (AMV-RT), T7 RNA polymerase and RNase H. In this reaction, RNA sequences serve as template for the extension of primer 1, which contains the T7-RNA polymerase recognition site, by AMV-RT. Extension is followed by degradation of the template RNAs by RNase H, synthesis of the second DNA strand through extension of primer 2 by AMV-RT, and RNA synthesis by T7 RNA polymerase. With RNA synthesis the system enters the isothermal cyclic phase, resulting in the accumulation of RNA amplificates. Three internal calibrators are co-amplified with the target sequence, and the amplified products are detected by electrochimioluminescence after hybridization with ruthenium-labeled probes [van Gemen et al., 1993a,b].

In the present study, we evaluated the Amplicor, bDNA and NASBA procedures for HIV-1 RNA quantitation in plasma specimens.

MATERIALS AND METHODS

Plasma Specimens

Ninety-four blood samples were collected on EDTA anticoagulant from 74 subjects, after informed consent; these subjects belonged to three groups. The first group consisted of 25 consecutively enrolled HIV-1 infected patients at different stages of HIV-1 infection. One blood specimen was studied for each of these patients; nine received antiretroviral therapy at the time of sampling. The second group consisted of 15 HIV-1 infected patients followed up after initiation of antiviral therapy: one was treated with zidovudine, six were treated with zidovudine plus didanosine, and eight were treated with foscarnet. A total of 35 samples were obtained from this group of patients; The first sample was collected before initiation of treatment and at least one other sample was collected during the course of the treatment. The third group included 34 HIV-1- and HIV-2-seronegative volunteer blood donors. Blood samples were centrifuged within 2 hr after venipuncture; plasma was then divided into aliquots and stored at −80°C until use.

HIV-1 Strains

Viral isolates representing HIV-1 subtypes A to H were cultured in phytohemagglutinin-stimulated peripheral blood mononuclear cells (2×10^6 cells per ml) from HIV-1- and HIV-2-seronegative donor. The strains were DJ258 and DJ263 (subtype A), IIIB and SF2 (subtype B), ZAM18 and ZAM20 (subtype C), UG270 and UG274 (subtype D), CM235 and CM241 (subtype E), 162–3069 and 163–3070 (subtype F), G98 and LBV21-7 (subtype G), and VI557 (subtype H). Supernatant cultures were diluted with a pool of plasmas from blood donors and stored at −80°C until testing.

HIV-1 RNA Quantitation

Amplicor, bDNA, and NASBA assays, carried out according to the manufacturers' instructions, are briefly described below:

(i) Amplicor. RNA was extracted from 0.2 ml plasma by using a lytic reagent containing guanidine thiocyanate (GuSCN) and a known amount of quantitation standard (QS). QS is a synthetic RNA molecule that contains primer binding sites identical to the HIV-1 target and a unique sequence that serves as target for a QS-specific probe. HIV-1 and QS RNA molecules were precipitated with isopropanol and resuspended in a buffer containing carrier RNA. A 142-base pair sequence in the gag gene of HIV-1 and QS were co-amplified with 5' end-biotinylated primers SK431 and SK462. Reverse transcription and PCR were carried out in a single reaction by using the thermostable recombinant enzyme rTth DNA polymerase. dUTP was incorporated into each amplification to serve as substrate for AmpErase enzyme (uracil N-glycosylase) to prevent carry-over contamination of previously ampli-

fied material. The biotinylated HIV-1 and QS amplicons were captured in separate wells of a microtiter plate coated with HIV-1-specific and QS-specific oligonucleotide probes, respectively. To quantify the amplicons over a large dynamic range, five-fold serial dilutions of HIV-1 and QS amplicons were made in the HIV-1-specific and QS-specific wells. The bound biotinylated amplicons were detected with an avidin-horseradish peroxidase conjugate and a chromogenic substrate mixture. Absorbance was measured at 450 nm. The HIV-1 RNA copy number was calculated from the known input copy number of the QS RNA.

(ii) bDNA. HIV-1 virions were pelleted from duplicate 1.0 ml plasma samples by centrifugation at 23,500 × *g* for 1 hr. Pellets were resuspended in a buffer containing proteinase K, lithium lauryl sulfate, and target probes complementary to the HIV-1 pol gene sequences. Hybridization of target probes with HIV-1 RNA was obtained by incubation at 53°C for 20 min. The mixture was then centrifuged at 23,500 × *g* for 15 min. A 0.2 ml aliquot of the clarified extract was transferred to microwells coated with capture probe complementary to a sequence of the target probes. The RNA-target probe complex was captured on the surface of the microwells during incubation overnight at 53°C. The wells were washed and the DNA amplifier molecules were hybridized to the captured target-probe complex at 53°C for 30 min. After subsequent washing, alkaline phosphate-labeled probes were hybridized with the amplifier bDNA molecules at 53°C for 15 min. After additional washes, the immobilized complex was incubated with a chemiluminescent substrate (dioxetane), and light emission was measured in a luminometer. The amount of HIV-1 RNA in each specimen was determined by reference to a standard curve, run in parallel with the patient specimens, and that consisted of a DNA plasmid at four concentrations. With this method, the results are expressed as the number of HIV-1 RNA equivalents per ml. With the aim of rendering the results obtained by the different procedures uniform, we considered that one HIV-1 RNA equivalent was equal to one HIV-1 RNA copy.

(iii) NASBA. HIV-1 RNA was extracted from either 0.1 or 1.0 ml of plasma by using a lysis buffer containing GuSCN. Three synthetic RNA calibrators (Qa, Qb, Qc) of known high, medium, and low concentration, respectively, were then added; each RNA calibrator contained a short unique sequence differing from the HIV-1 wild type. RNA was extracted by using acidified silica, according to the procedure described by Boom et al. [1990]. HIV-1 and calibrator RNA sequences were co-amplified by incubation of the extraction product with the primers and the enzyme mixture at 41°C for 90 min. The RNA concentration of HIV-1, Qa, Qb, and Qc amplificates was measured in four separated aliquots. In each aliquot, the amplified RNA was captured with a biotin-labeled oligonucleotide bound to streptavidin-coated magnetic beads acting as the solid phase, and then hybridized with a specific ruthenium-labeled probe complementary to the HIV-1-, Qa-, Qb-, or Qc-specific sequence. The magnetic beads carrying the hybridized amplificate-probe complex were captured on the surface of an electrode by means of a magnet. Voltage applied to the electrode triggered the electroluminescence reaction. The light emitted by the ruthenium-labeled complex was proportional to the amount of amplificate. Calculation of the relative amount of the four amplificates revealed the amount of HIV-1 RNA in the sample.

The main technical characteristics of Amplicor, bDNA, and NASBA are summarized in Table I.

Reproducibility Determination

Plasma specimens were prepared by diluting the HIV-1 III-B strain culture supernatant with a pool of plasma samples obtained from blood donors. Two HIV-1 RNA levels were tested for reproducibility. One high-level plasma specimen was used to test the reproducibility of the three quantitation procedures. The low-level plasma specimen was a 1:100 dilution of the high-level specimen in the Amplicor and NASBA procedures, and a 1:10 dilution of the high-level specimen in the bDNA procedure. Plasma specimens were tested five times per run in five separate runs for each lot, and two different lots were studied for each procedure. For NASBA reproducibility determination, the high-level plasma specimen was assayed by using 0.1 ml samples, whereas the low-level plasma specimen was assayed by using 1.0 ml samples. The coefficients of variation (CV) were calculated to determine the within-run, inter-run, and inter-lot reproducibility for the high-level and low-level specimens.

HIV-1 p24 Antigenemia

HIV-1 p24 antigen level in plasma was determined after acid dissociation of immune complexes by the Coulter HIV-1 p24 antigen assay (Coultronics, Margency, France). Quantitation was carried out using a standard curve. A cut-off of 30 μ/ml was set for this assay.

HIV-1 Subtyping

HIV-1 was isolated from patients' peripheral blood mononuclear cells following standard lymphocyte coculture [Jackson et al., 1990]. HIV-1 DNA was extracted from cultured cells by using the isoQuick isolation kit (Microprobe Corp., Garden Cove, CA). Subtyping was performed by the heteroduplex mobility assay [Delwart et al., 1993]. Briefly, DNA amplification by PCR involved a series of reactions, with 3 μl of the first round reaction product added to a second round of PCR with internally annealing primers. First round primers were ED5 and ED12, and second round primers were ES7 and ES8. Second round amplification yielded a product of approximately 700 bp, spanning the V3–V5 region of the HIV-1 envelope. For the first round the PCR conditions were as follows: 1 cycle at 94°C for 5 min, 55°C for 30 sec, and 72°C for 2 min, followed by 29 cycles at 94°C for 15 sec, 55°C for 30 sec, and 72°C for 2 min with the final extension at 72°C for 7 min. The conditions for the second round were: 1 cycle at 94°C for 5

TABLE I. Technical Characteristics of Amplicor, bDNA, and NASBA

Assay	Plasma volume (ml)	Lower detection limit (log copies/ml)	Number of samples per run	Run time (hour)
Amplicor	0.2	2.60	12	6
bDNA	2 × 1.0	4.00	42	24
NASBA	0.1 or 1.0	2.60[a]	10	6

[a]Lower detection limit for a 1.0 ml plasma sample. This value was 3.60 log copies/ml for a 0.1-ml plasma sample.

TABLE II. Reproducibility of Amplicor, bDNA, and NASBA for Quantitation of HIV-1 RNA

				Amplicor			bDNA			NASBA		
RNA level	Determination	Lot	Run	Mean copies/ml	SD	CV (%)	Mean copies/ml	SD	CV (%)	Mean copies/ml	SD	CV (%)
High	Whithin run	1	1	637,780	205,604	32	265,100	43,888	17	1,500,000	331,662	22
			2	355,932	40,756	11	278,120	29,848	11	1,366,000	355,358	26
			3	314,623	94,235	30	290,640	16,704	6	1,140,000	167,332	15
			4	267,463	64,020	24	285,400	28,919	10	1,680,000	414,728	25
			5	463,573	56,540	12	376,060	22,198	6	1,282,000	291,753	23
		2	1	331,019	49,582	15	326,600	61,159	19	2,640,000	403,732	15
			2	410,448	52,804	13	431,680	32,653	8	2,460,000	912,688	37
			3	558,146	114,786	21	332,620	53,553	16	2,500,000	355,902	14
			4	592,565	86,439	15	382,680	69,560	18	2,340,000	403,732	17
			5	322,099	72,734	23	455,920	58,575	13	2,720,000	342,052	13
	Inter-run	1		398,295	158,587	40	299,064	48,558	16	1,393,000	349,998	25
		2		442,855	36,009	31	385,900	73,767	19	2,533,333	507,908	20
	Inter-lot			421,030	146,156	35	342,482	75,025	22	1,951,836	718,495	37
Low	Whithin run	1	1	3,924	674	17	47,954	12,443	26	9,140	3,642	40
			2	2,052	560	27	58,308	3,236	7	11,280	7,040	62
			3	2,834	443	16	52,080	13,934	27	11,200	3,346	30
			4	2,486	400	16	53,166	7,076	13	9,880	2,399	24
			5	4,566	682	15	75,826	21,028	28	8,500	4,964	58
		2	1	3,271	1,083	33	69,558	24,166	35	6,660	1,876	28
			2	2,745	858	31	84,496	6,620	8	9,720	4,778	49
			3	4,440	1,120	25	67,730	20,989	31	11,620	4,579	39
			4	4,381	923	21	77,654	6,946	9	8,120	4,203	51
			5	2,752	804	29	99,954	11,794	12	5,200	1,918	37
	Inter-run	1		3,172	1,082	34	55,467	15,945	29	10,000	4,321	43
		2		3,518	1,170	33	79,878	18,753	23	8,276	4,090	49
	Inter-lot			3,345	1,118	33	67,673	20,971	31	9,138	4,254	47

min, 55°C for 30 sec, and 72°C for 2 min, followed by 39 cycles at 94°C for 15 sec, 55°C for 30 sec, and 72°C for 2 min, with the final extension at 72°C for 7 min. The PCR amplification products were detected by electrophoresis on a 1% agarose gel. Heteroduplex molecules were obtained by mixing two divergent PCR amplified DNA fragments (the unknown patient strain with a plasmid from typed references strains) denatured at 96°C for 3 min and renatured by rapid cooling on ice. The reaction was performed in 100 mM NaCl, 10 mM Tris HCl (pH 7.8) and 2 mM EDTA in a final volume of 8 µl. The heteroduplex formation was resolved by electrophoresis analysis at 200 V for 3 h on a nondenaturing 5% polyacrylamide gel in TBE buffer (88 mM Tris borate, 89 mM boric acid, 2 mM EDTA) and was detected after staining with ethidium bromide. The electrophoretic mobility of the heteroduplexes was inversely proportional to the sequence divergence of the two annealed strands.

Data Analysis

Results were expressed as log HIV-1 RNA copies per ml of plasma. The number of HIV-1 RNA copies per ml were used for the calculation of the coefficients of variation (CV = S.D./mean). Statistical comparison of the means were performed by Student's *t* test. Values lower than the detection limit were omitted for the calculation of means. Significance tests of correlations were performed by the standard linear regression method. To evaluate the correlation between the HIV-1 RNA levels and either HIV-1 p24 antigenemia or CD4+ cell counts, specimens from the patients who had antiviral therapy at the time of testing were omitted. We considered as concordant results obtained by two methodologies for a given specimen when a log difference of <0.5 was observed; they were considered as highly discordant when the log difference was >1.0.

RESULTS

Specificity, Sensitivity, and Reproducibility

Among the 60 plasma specimens collected from HIV-1 infected subjects, HIV-1 RNA could be quantitated in 56 by Amplicor (sensitivity, 93.3%), in 41 by bDNA (sensitivity, 68.3%), and in 60 by NASBA (sensitivity, 100%).

Plasma samples collected from HIV-1-seronegative

TABLE III. HIV-1 RNA Levels Determined by Amplicor, bDNA, and NASBA in Plasma Specimens From HIV-1 -infected Patients

Patient no.	Disease stage	CD4+ cell count (cells/mm³)	HIV-1 p24 antigenemia (pg/ml)	HIV-1 subtype	HIV-1 RNA quantitation		
					Amplicor	bDNA	NASBA
Group 1							
1	A	456	<30	ND[a]	3.90	<4.00	3.26
2	B	260	<30	A	<2.60	<4.00	4.89
3	B	148	<30	B	5.14	5.17	5.39
4	A	502	ND	ND	3.89	<4.00	3.75
5	C	18	<30	B	4.70	<4.00	3.95
6	A	389	349	ND	5.04	4.88	5.08
7	B	448	ND	B	4.11	<4.00	4.28
8	A[b]	619	57	ND	5.64	4.92	5.60
9	C	58	34	B	5.74	5.40	5.34
10	A	284	<30	B	4.85	4.45	4.32
11	A	1038	<30	ND	5.17	4.08	4.92
12	B	34	110	B	5.16	4.69	4.86
13	A	707	<30	ND	4.04	<4.00	3.90
14	B	225	92	ND	4.52	4.36	4.43
15	A	491	200	B	4.90	4.30	4.81
16	A	280	77	B	4.67	4.52	4.57
17	A	403	ND	ND	4.00	<4.00	4.08
18	B	233	<30	ND	4.32	4.41	4.53
19	C	35	1140	B	6.52	6.04	5.94
20	B	442	166	B	5.12	4.85	5.36
21	B	76	ND	ND	5.58	4.95	5.59
22	A	367	<30	E	<2.60	<4.00	3.14
23	A	319	<30	ND	4.90	4.04	4.70
24	A	201	<30	ND	3.83	<4.00	3.72
25	A	432	<30	A	<2.60	4.04	4.43
Group 2, first determination							
1	C	348	1870	B	5.23	5.22	5.76
2	C	32	110	B	5.35	5.15	5.32
3	B	203	<30	ND	4.08	<4.00	4.15
4	C	50	538	ND	5.55	4.70	4.90
5	C	376	166	B	5.99	5.40	5.79
6	C	170	209	B	5.87	5.41	5.83
7	C	256	35	B	4.90	4.08	5.28
8	C	4	39	ND	6.0	5.82	6.40
9	C	153	253	B	5.94	6.00	6.00
10	C	1	<30	B	5.75	5.71	5.87
11	C	58	128	ND	5.40	5.13	5.40
12	C	60	153	B	5.53	5.24	5.45
13	C	2	79	B	5.86	5.38	6.04
14	C	2	122	B	5.98	5.57	5.94
15	C	331	<30	ND	5.25	4.83	5.29

[a]ND, not determined.
[b]Primary infection.

blood donors were all negative (i.e., copy number < detection limit) by Amplicor, bDNA, and NASBA, indicating that the three techniques had a specificity of 100%.

Data on the reproducibility of the three methods are shown in Table II. The within-run CVs ranged from 11 to 33% for Amplicor, from 6 to 35% for bDNA and from 13 to 62% for NASBA. In the high-level plasma specimen, the mean within-run CV was 19.6% for Amplicor, 12.4% for bDNA and 20.7% for NASBA; in the low-level specimen, the value was 23% for Amplicor, 19.6% for bDNA and 41.8% for NASBA. The inter-run CVs ranged from 31 to 40% for Amplicor, from 16 to 29% for bDNA and from 20 to 49% for NASBA. The inter-lot CVs ranged from 22 to 47%, according to the different methods. The lowest inter-lot CVs were obtained by bDNA. In the high-level plasma specimen, the inter-lot CVs obtained by Amplicor (35%) and NASBA (37%) were very similar; in the low-level plasma specimen, however, the inter-lot CV obtained by Amplicor (33%) was lower than the inter-lot CV obtained by NASBA (47%) and was very similar to the inter-lot CV obtained by bDNA (31%).

Copy Number Determination in the Plasma Specimens

When we considered the 40 specimens for which HIV-1 RNA levels were higher than the detection limit of each method, the mean HIV-1 RNA levels determined by Amplicor, bDNA and NASBA were 5.38 ± 0.52 log copies per ml, 5.03 ± 0.55 log copies per ml, and 5.39 ± 0.53 log copies per ml, respectively. The mean values significantly differed between Amplicor and bDNA ($P < 0.01$) and between NASBA and b-DNA ($P < 0.01$), whereas no significant difference was observed between Amplicor and NASBA ($P > 0.90$).

As shown in Table III, the HIV-1 RNA level could be

TABLE IV. Variation in the HIV-1 RNA Copy Number From Baseline in Patients Followed up After Initiation of Antiviral Therapy

Treatment	Patient No.	Number of days after initiation	Deviation of log HIV-1 RNA copy number[a]			
			Amplicor	bDNA	NASBA	Mean
AZT	3	56	<−1.78[b]	−[c]	−0.48	—
AZT-ddI	1	0[d]	+0.48	+0.25	−0.10	+0.21
		27	−2.23	<−0.80[b]	−3.06	−2.64
	4	29	−2.70	<−0.69[b]	−2.30	−2.50
	5	0[e]	−0.41	−0.10	+0.11	−0.13
		44	−2.08	<−1.40[b]	−1.41	−1.74
	9	32	−2.54	<−2.00	−2.07	−2.30
	11	0[f]	+0.57	+0.50	+0.50	+0.52
		15	−2.23	<−1.13[b]	−1.91	−2.07
	15	32	−1.85	<−0.83[b]	−2.41	−2.13
Foscarnet	2	6	+0.35	−0.33	−0.06	−0.01
	6	11	−1.57	−1.33	−0.95	−1.28
		20	−1.57	−1.21	−1.10	−1.29
		29	−0.82	−0.41	−0.30	−0.51
	7	15	−0.67	<−0.07	−1.10	−0.88
	8	14	−0.52	−0.67	−0.80	−0.66
	10	12	−0.34	−0.27	+0.07	−0.18
	12	18	−1.79	<−1.23[b]	−1.41	−1.60
	13	16	+0.11	+0.13	+0.16	+0.13
	14	77	−0.18	−0.27	−0.11	−0.18

[a]Results for the first sample are reported in Table III.
[b]HIV-1 RNA level below the lower limit of detection of the method; these results were omitted for calculation of the mean.
[c]Deviation could not be determined since the HIV-1 RNA level was below the lower limit of detection in both plasma specimens.
[d]The first plasma specimen was collected 4 days before initiation of therapy.
[e]The first plasma specimen was collected 29 days before initiation of therapy.
[f]The first plasma specimen was collected 6 days before initiation of therapy.

determined by NASBA in all the 25 plasma samples from the first group of patients, whereas this level could not be quantitated in three samples by Amplicor and in nine samples by bDNA. Among the 35 samples sequentially collected in the second group of patients, the HIV-1 RNA level could be determined in all the samples by NASBA, but could not be determined in one sample by Amplicor and in 10 samples by bDNA. When we compared the HIV-1 RNA levels after initiating antiviral therapy in the second group of patients (Table IV), we observed that in all patients but one (patient no. 3), the three methods gave very similar changes in RNA levels. In patient no. 3, a pregnant women treated with zidovudine, a greater decrease was observed by Amplicor in comparison with NASBA (>1.78 log vs. 0.48 log). Among the 15 patients followed up after initiation of antiviral therapy, the HIV-1 RNA level variation could not be determined precisely in one patient by Amplicor and in eight patients by bDNA since RNA levels dropped below the detection limit.

Correlation Between Amplicor, bDNA, and NASBA

As shown in Figure 1, a significant linear relationship was observed between the HIV-1 RNA levels as determined by the different pairs of methods. We also compared the frequency of concordant and discordant results between the different methods. The results, presented in Table V, show that the highest concordance was observed between Amplicor and NASBA, and the lowest concordance between bDNA and NASBA.

Relationship to HIV-1 P24 Antigenemia

As shown in Table VI, significant differences in RNA levels, quantitated by Amplicor, bDNA, and NASBA, were observed between patients with positive or negative HIV-1-p24 antigenemia. However, there was no direct correlation between the HIV-1 P24 antigenemia level and the HIV-1 RNA level as determined by Amplicor ($r = 0.19$, $N = 30$, $P > 0.1$), bDNA ($r = 0.21$, $N = 24$, $P > 0.1$), and NASBA ($r = 0.29$, $N = 33$, $P > 0.1$).

Relationship to CD4+ T Cell Count

Patients with <200 CD4+ T cells per mm^3 had significantly higher levels of HIV-1 RNA in plasma than patients with >200 CD4+ T cells per mm^3 (Table VI). We also observed a negative correlation between the CD4+ T cell count and the HIV-1 RNA level as determined by Amplicor ($r = -0.45$, $N = 30$, $P < 0.01$), b-DNA ($r = -0.63$, $N = 24$, $P < 0.01$), and NASBA ($r = -0.35$, $N = 33$, $P < 0.05$).

Effect of HIV-1 Subtype

Among patients of the first group, three were infected by a non-B HIV-1 subtype strain: two subtype A and one subtype E strains. As shown in Table III, the corresponding plasma samples could be quantitated by NASBA but could not be quantitated by Amplicor

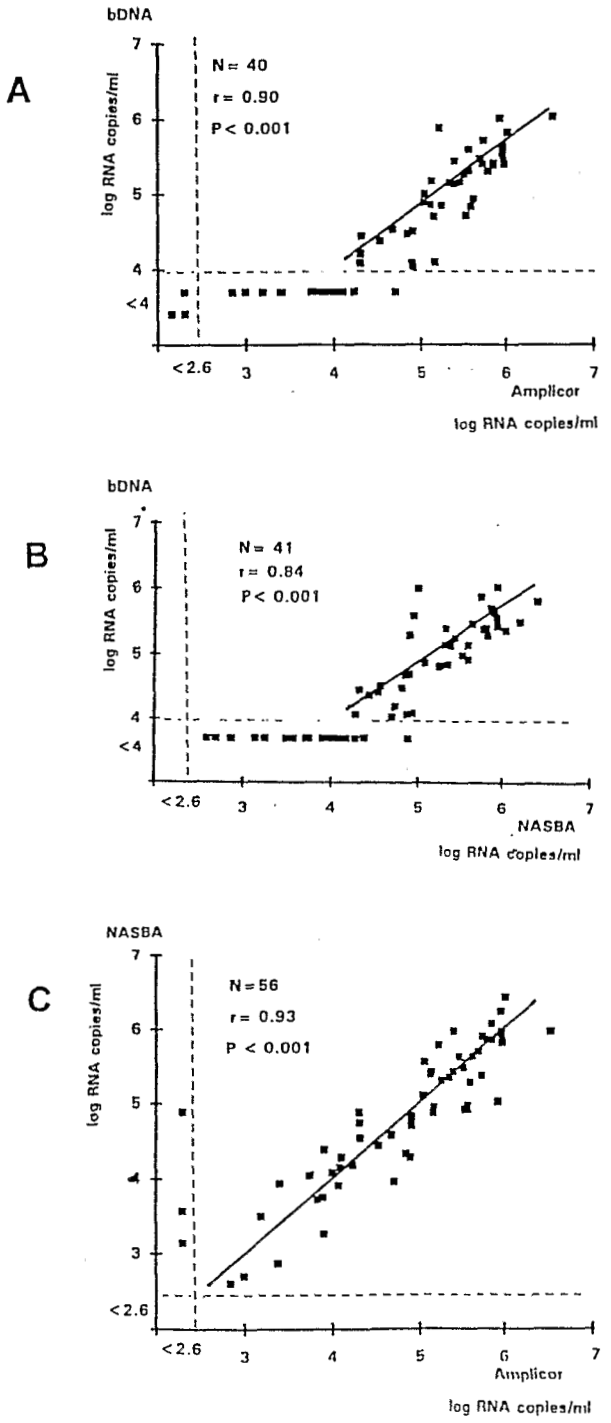

Fig. 1. Correlation between Amplicor and bDNA (A), bDNA and NASBA (B), and Amplicor and NASBA (C). Dashed lines represent the lower limits of detection.

whereas, for two of these plasma specimens, the RNA level was below the bDNA detection limit. We therefore tested different HIV-1 subtype strains diluted with a pool of plasma obtained from HIV-1-seronegative blood donors. Differences were observed according to the strains tested (Table VII): subtype A strains were not detected by Amplicor. We also observed that the copy number detected by Amplicor was much lower than the copy number detected by bDNA (log difference >1.0) in one subtype E and one subtype F strain. Furthermore, subtype G HIV-1 RNA could not be detected by NASBA. On the other hand, RNA from all the HIV-1 subtypes could be detected and quantitated by bDNA. However, lower HIV-1 RNA levels were measured by bDNA than by Amplicor and NASBA for subtype B strains.

DISCUSSION

HIV-1 RNA level in plasma now constitutes a primordial criteria for the therapeutic decision in HIV-1 infection. Therefore, a specific, sensitive, and reproducible procedure must be used for this purpose. In the present study, we compared the performances of the three currently available commercial procedures for HIV-1 RNA quantitation in plasma, namely, Amplicor, bDNA, and NASBA.

HIV-1 RNA could not be detected in the plasma samples of noninfected subjects. Therefore, the three methodologies were 100% specific. Differences were observed in sensitivity, since HIV-1 RNA could not be detected by Amplicor and bDNA in respectively 6.7% and 31.7% of the plasma specimens collected in HIV-1-infected subjects. However, HIV-1 RNA could be quantitated in all these specimens by using NASBA. The defect of sensitivity observed with bDNA can be explained by the high detection limit of this method (4.0 log HIV-1 RNA copies per ml), whereas Amplicor and NASBA have similar detection limits that are more than 20 times lower than the bDNA detection limit.

The results indicate that bDNA is the most reproducible method. In the high-level plasma specimen, the reproductibilities of Amplicor and NASBA were not very different since the mean within-run CVs were about 20% and the inter-lot CVs about 35%. In the low-level plasma specimen, however, the reproducibility of Amplicor was not very different from the reproducibility of bDNA since the mean within-run CVs were about 20% and the inter-lot CVs about 30%, whereas the reproducibility of NASBA was lower, as shown by CVs higher than 40%.

It was observed that the genetic variability of HIV-1 affects the quantitation of HIV-1 RNA by the different techniques. Indeed, we observed that HIV-1 RNA could not be detected by Amplicor, neither in the plasma specimens from subtype-A infected patients, nor in the culture supernatants of subtype A references strains. The lack of detection of subtype A HIV-1 strains by Amplicor has already been reported [Todd et al., 1994; Loussert-Ajaka et al., 1995]. We also observed that HIV-1 RNA could not be detected by NASBA in the culture supernatants of subtype G HIV-1 references strains. Moreover, in a defined HIV-1 subtype, inter-strain genetic variability could also affect the determination of the RNA level since differences were observed between subtype E or subtype F strains by using Amplicor. On the other hand, HIV-1 RNA from all the subtype reference strains could be quantitated by bDNA. Both Amplicor and NASBA methodologies are based on amplification of a target sequence by using a couple of primers, and the

TABLE V. Differences Between HIV-1 RNA Levels Determined by Amplicor, bDNA, and NASBA

Methods compared (no. of specimens)[a]	log difference	Number	% (95% CI)
Amplicor-bDNA (40)	<0.50[b]	31	77.5 (64.6–90.4)
	0.50–0.99[c]	8	37.5 (7.6–32.4)
	≥1.0[d]	1	2.5 (0.0–7.4)
Amplicor-NASBA (58)	<0.05	45	80.4 (70.0–90.8)
	0.50–0.99	11	19.6 (9.2–30.0)
	≥1.0	0	0.0 (0.0–3.0)
bDNA-NASBA (41)	<0.50	24	58.6 (43.6–73.6)
	0.50–0.99	16	39.0 (24.1–53.9)
	> 1.0	1	2.4 (0.0–3.0)

[a]Specimens with RNA level < detection limit were omitted.
[b]Results differing by <0.05 log are considered as concordant.
[c]Results differing by 0.50–0.99 log are considered as discordant.
[d]Results differing by ≥1.0 log are considered as highly discordant.

TABLE VI. Relationship Between RNA Levels, HIV-1 p24 Antigenemia, and CD4+ T Cell Counts

Method	Antigenemia (pg/ml)			CD4+ T cell count (number/mm^3)		
	<30	>30	*P*	<200	>200	*P*
Amplicor	4.61 ± 0.69	5.42 ± 0.54	<.01	5.66 ± 0.36	4.87 ± 0.69	<.01
bDNA	4.63 ± 0.61	5.20 ± 0.42	<.05	5.43 ± 0.35	4.79 ± 0.46	<.01
NASBA	4.35 ± 0.73	5.50 ± 0.53	<.001	5.43 ± 0.80	4.80 ± 0.75	<.05

TABLE VII. Effect of the HIV-1 Subtype on Quantitation of HIV-1 RNA by Amplicor, bDNA, and NASBA

HIV-1 strain	Subtype	HIV-1 RNA level (copies per ml)		
		Amplicor	bDNA	NASBA
DJ258	A	<2.60	5.05	5.00
DJ263	A	<2.60	4.90	4.78
SF2	B	5.35	4.58	5.38
HI-B	B	4.73	4.23	5.56
ZAM18	C	4.89	4.85	4.82
ZAM20	C	5.25	5.10	5.62
UG270	D	5.25	4.47	5.23
UG274	D	5.50	4.62	4.51
CM241	E	4.27	4.86	4.54
CM235	E	3.67	4.72	4.18
163.3069	F	4.56	4.97	4.76
162.3070	F	3.45	4.89	4.41
G98	G	5.40	5.43	<2.60
LBV21	G	5.27	5.47	<2.60
VI557	H	5.98	5.77	5.10

amplified product is thereafter detected by using a probe complementary to a unique sequence. As expected, these two procedures were more affected by the genetic variation of HIV-1 than bDNA, which is based on hybridization of several probes with different sequences along the pol gene of HIV-1. In HIV-1 subtype B reference strain culture supernatants, however, RNA levels obtained by bDNA were lower than those obtained by Amplicor or NASBA. This observation is in accordance with the results obtained for our clinical specimens (95% subtype B) since compared with Amplicor and NASBA, lower HIV-1 RNA levels were determined by bDNA. These results suggest that the HIV-1 RNA levels in plasma from patients infected with subtype B HIV-1 were possibly underestimated by bDNA. However, since it has been reported that bDNA is not affected by genetic variability [Todd et al., 1994, 1995], the possibility of an overestimation of subtype B HIV-1 RNA levels by the target sequence amplification-based procedures such as Amplicor and NASBA cannot be excluded.

Taken together, these data indicate that bDNA is a highly reproducible method that allows quantitation of RNA from different HIV-1 subtypes. However, bDNA has a low sensitivity since, in our experience, HIV-1 RNA could not be quantitated in more than 30% of the patient specimens. In particular, HIV-1 RNA could not

be quantitated by bDNA in 7/7 patients followed up after initiation of antiretroviral treatment with zidovudine or zidovudine plus didanosine. Therefore, the presently available bDNA assay cannot be used for an effective follow-up of HIV-1 infected patients with low HIV-1 RNA levels. However, according to the manufacturer, a bDNA assay with improved sensitivity (detection limit <3.0 log HIV-1 RNA copies per ml) is being developed. On the other hand, Amplicor and NASBA, both based on target sequence amplification, presented comparable performances. These methods were much more sensitive than bDNA, but they were less reproducible. The use of Amplicor and NASBA can be restricted by HIV-1 genetic variability since HIV-1 RNA from strains belonging to certain African HIV-1 subtypes were not detected by Amplicor or NASBA. In particular, Amplicor cannot be used in patients infected with subtype A HIV-1 strains, whereas NASBA cannot be used in patients infected with subtype G HIV-1 strains.

A significant correlation was found between the HIV-1 RNA levels as determined by the different procedures. The best correlation was observed between the results obtained by Amplicor and NASBA. The highest proportion of concordant results (i.e., log difference < 0.50) was also observed with Amplicor and NASBA, whereas the lowest proportion of concordant results was observed between bDNA and NASBA. Therefore, Amplicor and Nasba, in addition to the fact that they are both based on sequence amplification, can be considered as closely related procedures.

Similar results were obtained with Amplicor, bDNA, and NASBA when the HIV-1 RNA levels were compared with the CD4+ T cell counts or the HIV-1-p24 antigen levels. The inverse correlation observed between HIV-1 RNA levels and CD4+ cell counts, as well as the lack of correlation between HIV-1 RNA and HIV-1-p24 antigen levels, confirm previously reported results [Winters et al., 1993; Lin et al., 1994; van Kerckhoven et al., 1994]. These data indicate that the HIV-1 RNA level in plasma, which reflects HIV-1 replication in lymphoid organs, is correlated with immune impairment. Therefore, the CD4+ cell count and the HIV-1 RNA level in plasma constitute complementary markers which can be associated advantageously. The higher sensitivity of HIV-1 RNA detection in plasma, in comparison with HIV-1 p24 antigen detection [van Kerckhoven et al., 1994], as well as the lack of correlation between HIV-1 RNA and HIV-1 p24 levels question the usefulness of HIV-1 p24 antigenemia for monitoring HIV-1 infected patients.

Similar variations in HIV-1 RNA levels, as determined by Amplicor, bDNA, and NASBA, were observed after initiation of antiviral treatment. The only patient in which a marked difference in the RNA level decrease was observed had a low baseline level. We therefore suppose that a reduced accuracy of the methods with RNA levels near the detection limit could explain this difference. Our results confirm the high decrease (about 2.0 log) of the HIV-1 RNA level in patients treated with the combination of zidovudine and didanosine [Shafer et al., 1995]. It has been reported that foscarnet, an anti-cytomegalovirus drug, has antiretroviral activity in vitro [Koshida et al., 1989] and in vivo [Reddy et al., 1992]. It has also been shown that foscarnet therapy decreases HIV-1 RNA level in plasma [Kaiser et al., 1995]. In the present study, HIV-1 RNA levels were sequentially determined by Amplicor, bDNA, and NASBA in patients who were treated with foscarnet for a cytomegalovirus disease. The results obtained by the three methodologies are concordant and confirm the previously observed anti-HIV-1 activity of foscarnet. However, in a high proportion of patients treated with antiretroviral drugs, decreases in HIV-1 RNA levels could not be precisely determined by using bDNA since RNA levels frequently fell below the detection limit, indicating that Amplicor and NASBA are more convenient procedures than bDNA for the follow up of patients undergoing antiretroviral therapy.

In a recent report, Revets et al. [1996] compared the three HIV-1 RNA quantitation procedures and concluded that the three assays can be used to measure the HIV-1 RNA copy number. We agree with this conclusion. However, our study clearly shows that the performances of the presently available tests could be improved either by decreasing the detection limit (bDNA) or by quantitating more efficiently the non-B HIV-1 strains (Amplicor and NASBA).

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REFERENCES

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, Van der Noordaa J (1990): Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28:495–503.

Cao Y, Qin L, Zhang L, Safrit J, Ho DD (1995): Virologic and immunologic characterization of long term survivors of human immunodeficiency virus type 1 infection. New England Journal of Medicine 332:202–208.

Cohen OJ, Pantaleo G, Holodniy M, Schnittman S, Niu M, Graziosi C, Pavlakis GN, Lalezari J, Bartletti JA, Steigbigel RT, Cohn J, Novak R, McMahon D, Fauci AS (1995): Decreased human immunodeficiency virus type 1 plasma viremia during antiretroviral therapy reflects downregulation of viral replication in lymphoid tissue. Proceedings of the National Academy of Sciences of the USA 92:6017–6021.

Delwart EL, Shpaer EG, Louwagie J, McCutchan FE, Grez M, Rübsamen-Waigmann H, Mullins JI (1993): Genetic relationships determined by a DNA heteroduplex mobility assay: Analysis of HIV-1 env genes. Science 262:1257–1261.

Dewar RL, Highbarger HC, Sarmiento MD, Todd JA, Vasudevachari MB, Davey RT, Kovacs JA, Salzman NP, Lane HC, Urdea MS (1994): Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. Journal of Infectious Diseases 170:1172–1179.

Henrard DR, Phillips JF, Muenz LR, Blattner WA, Wiesner D, Eyster ME, Goedert JJ (1995): Natural history of HIV-1 cell-free viremia. Journal of the American Medical Association 274:554–558.

Hogervorst E, Jurriaans S, de Wolf F, van Wijk A, Wiesma A, Valk M, Roos M, van Gemen B, Coutinho R, Miedema F, Goudsmit J (1995): Predictors for non- and slow progression in human immunodefi-

ciency virus (HIV) type 1 infection: Low viral RNA copy numbers in serum and maintenance of high HIV-1 p24-specific but not V3-specific antibody levels. Journal of Infectious Diseases 171: 811–821.

Holodniy M, Katzenstein DA, Sengupta S, Wang AM, Casipit C, Schwartz DH, Konrad M, Groves E, Merigan TC (1991): Detection and quantification of human immunodeficiency virus RNA in patient serum by use of the polymerase chain reaction. Journal of Infectious Diseases 163:862–866.

Jackson JB, Kwok SY, Sninsky JJ, Hopsicker JS, Sannerud KJ, Rhame FS, Henry K, Simpson M, Balfour (Jr) HH (1990): Human immunodeficiency virus detected in all seropositive symptomatic and asymptomatic individuals. Journal of Clinical Microbiology 28:16–19.

Kaiser L, Perrin L, Hirschel B, Furrer H, Von Overbeak J, Olmain M, Yerly S (1995): Foscarnet decreases human immunodeficiency virus RNA. Journal of Infectious Diseases 172:225–227.

Koshida R, Vrang L, Gilljam G, Harmemberg J, Oberg B, Wahren B (1989): Inhibition of human immunodeficiency virus in vitro by combination of 3'-azido-3'-deoxy-thymidine and foscarnet. Antimicrobials Agents and Chemotherapy 33:778–780.

Lin HJ, Myers LE, Yen-Lieberman B, Hollinger FB, Henrard D, Hooper CJ, Kokka R, Kwok S, Rasheed S, Vahey M, Winters MA, McQuay LJ, Nara PL, Reicherderfer P, Coombs RW, Jackson JB (1994): Multicenter evaluation of quantitation method for plasma human immunodeficiency virus type 1 RNA. Journal of Infectious Diseases 170:553–562.

Loussert-Ajaka I, Descamps D, Simon F, Brun-Vézinet F, Ekwalanga M, Saragosti S (1995): Genetic diversity and HIV detection by polymerase chain reaction. Lancet 346:912–913.

Loveday C, Kaye S, Tenant-Flowers M, Semple M, Ayliffe V, Weller IVD, Tedder RS (1995): HIV-1 RNA serum-load and resistant viral genotypes during early zidovudine therapy. Lancet 345:820–824.

Mellors JW, Kingsley LA, Rinaldo CR, Todd JA, Hoo BS, Kokka RP, Gupta P (1995): Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. Annals of Internal Medicine 122:573–579.

Mellors JW, Rinaldo CR, Gupta P, White RM, Todd JA, Kingsley LA (1996): Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272;1167–1170.

Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S (1994): Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. Journal of Clinical Microbiology 32:292–300.

O'Brien WA, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S, Rubin M, Simberkoff MS, Hamilton JD, and the Veterans Affairs Cooperative Study Group on AIDS (1996): Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. New England Journal of Medicine 334:426–431.

Pachl C, Todd JA, Kern DG, Sheridan PJ, Fong S-J, Stempien M, Hoo B, Besemer D, Yeghiazarian T, Irvine B, Kolberg J, Kokka R, Neuwald P, Urdea MS (1994): Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology 8:446–454.

Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, Cohen OJ, Desmarest JF, Montefiori D, Orenstein JM, Fox C, Schrager LK, Margolick JB, Buchbinder S, Giorgi JV, Fauci AS (1995): Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. New England Journal of Medicine 332:209–216.

Piatack (Jr) M, Saag MS, Yang LC, Clark SJ, Kappes JC, Luk K-C, Hahn BH, Shaw GM, Lifson JD (1993): High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 259:1749–1754.

Reddy MM, Grieco MH, McKinley GF, Causey DM, van der Horst CM, Parenti DM, Hooton TM, Davis RB, Jacobson MA (1992): Effect of foscarnet therapy on human immunodeficiency virus p24 antigen levels in AIDS patients with cytomegalovirus retinitis. Journal of Infectious Diseases 166:607–610.

Revets H, Marissens D, De Wit S, Lacor P, Clumeck N, Lauwers S, Zissis G (1996): Comparative evaluation of NASBA HIV-1 RNA QT, AMPLICOR-HIV Monitor, and QUANTIPLEX HIV RNA Assay, three methods for quantification of human immunodeficiency virus type 1 RNA in plasma. Journal of Clinical Microbiology 34:1058–1064.

Schuurman R, Nijhuis M, van Leeuwen R, Schipper P, de Jong D, Collis P, Danner SA, Mulder J, Loveday C, Christopherson C, Kwok S, Sninsky J, Boucher CAB (1995): Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus population in persons treated with lamivudine (3TC). Journal of Infectious Diseases 171:1411–1419.

Semple M, Loveday C, Weller I, Tedder R (1991): Direct measurement of viraemia in patients infected with HIV-1 and its relationship to disease progression and zidovudine therapy. Journal of Medical Virology 35:38–45.

Shafer RW, Iversen AKN, Winters MA, Aguiniga E, Katzenstein DA, Merigan TC, and the AIDS Clinical Trials Group 143 Virology Team (1995): Drug resistance and heterogeneous long-term virologic responses of human immunodeficiency virus type 1-infected subjects to zidovudine and didanosine combination therapy. Journal of Infectious Diseases 172:70–78.

Todd J, Pachl C, White R, Yeghiazarian T, Johnson P, Taylor B, Holodniy M, Kern D, Hamren S, Chernoff D, Urdea M (1995): Performance characteristics for the quantitation of plasma HIV-1 RNA using branched DNA signal amplification technology. Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology 10 (suppl. 2)S35–S44.

Todd J, Yeghiazarian T, Hoo B, Detmer J, Kolberg J, White R, Wilber J, Urdea M (1994): Quantitation of human immunodeficiency virus plasma RNA by branched DNA and reverse transcription coupled polymerase chain reaction assay method: a critical evaluation of accuracy and reproducibility. Serodiagnosis and Immunotherapy in Infectious Disease 6:233–239.

van Gemen B, Kievits T, Nara P, Huisman HG, Jurriaans S, Goudsmit J, Lens P (1993a): Qualitative and quantitative detection of HIV-1 RNA by nucleic acid sequence-based amplification. AIDS 7(suppl. 2):S107–S110.

van Gemen B, Kievits T, Schukkink R, van Strijp D, Malek LT, Sooknanan R, Huisman HG, Lens P (1993b): Quantification of HIV-1 RNA in plasma using NASBA during HIV-1 primary infection. Journal of Virological Methods 43:177–187.

van Kerckhoven I, Fransen K, Peeters M, de Beenhouwer H, Piot P, van der Groen G (1994): Quantitation of human immunodeficiency virus in plasma by RNA PCR, viral culture, and p24 antigen detection. Journal of Clinical Microbiology 32:1669–1673.

Verhofstede C, Reniers S, van Wanzeele F, Plum J (1994). Evaluation of proviral copy number and plasma RNA level as early indicators of progression in HIV-1 infection: correlation with virological and immunological markers of disease. AIDS 8:1421–1427.

Winters MA, Tan LB, Katzenstein DA, Merigan TC (1993): Biological variation and quality control of plasma human immunodeficiency virus type 1 RNA quantitation by reverse transcriptase polymerase chain reaction. Journal of Clinical Microbiology 31:2960–2966.