JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1998, p. 3372–3374 Vol. 36, No. 11
0095-1137/98/$04.00+0


# Evaluation of the Nuclisens HIV-1 QT Assay for Quantitation of Human Immunodeficiency Virus Type 1 RNA Levels in Plasma

MICHEL SEGONDY,[1]* THOAI-DUONG LY,[2] MARTINE LAPEYRE,[2] AND BRIGITTE MONTES[1]

*Laboratoire de Virologie, Centre Hospitalier Universitaire de Montpellier, Montpellier,*[1] *and Institut Fournier, Paris,*[2] *France*

Received 20 March 1998/Accepted 10 August 1998

**Nuclisens HIV-1 QT is a new version of the NASBA HIV-1 QT assay for quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma. The specificity of this assay was 100% in one laboratory and 99%—with nonrepeatability of the initial false positive—in another. The test was linear between 2.0 and 6.0 log RNA copies per ml. According to the input HIV-1 RNA concentration, accuracy varied from −0.11 to +0.10 log RNA copy per ml and precision varied from 0.66 to 0.14 log RNA copy per ml. Reproducibility decreased when the HIV-1 RNA level was near the lower limit of quantitation of the test. HIV-1 RNA could be quantitated by Nuclisens HIV-1 QT in 36% (laboratory 1) and 24% (laboratory 2) of clinical samples with HIV-1 RNA levels lower than the lower limit of quantitation by NASBA HIV-1 QT. Nuclisens HIV-1 QT was not suitable for measurement of RNA from clade G and group O HIV-1 strains.**

Human immunodeficiency virus type 1 (HIV-1) RNA level in plasma (viral load) is a marker of major importance for the follow-up and management of HIV-1-infected patients. Indeed, an increase in the HIV-1 RNA level in plasma predicts disease progression, whereas in patients treated with antiretroviral drugs, a decrease in the HIV-1 RNA level in plasma indicates treatment efficacy (1, 3, 6, 11). Therefore, the HIV-1 RNA level in plasma is regularly determined for patients undergoing antiretroviral therapy. The commercially available methods for quantitation of HIV-1 RNA in plasma are based on quantitative reverse transcription-PCR assay (7), nucleic acid sequence-based amplification (NASBA) (10), or branched DNA (4); the three methods demonstrated equal reliability (2, 8, 9). The current lower limits of quantitation by these assays are 200, 400, and 500 HIV-1 RNA copies/ml for reverse transcription-PCR, NASBA, and branched DNA, respectively. However, in patients with multidrug combination therapies, HIV-1 RNA levels in plasma frequently drop below these limits. Therefore, there is a need for the development of new assays with increased sensitivity for quantitation of low HIV-1 RNA levels in plasma. Nuclisens HIV-1 QT (Organon Teknika, Boxtel, The Netherlands) is an assay based on the NASBA methodology which presents a lower limit of quantitation than the first-generation NASBA HIV-1 QT assay (80 versus 400 HIV-1 RNA copies/ml). In this study, we evaluated the specificity, reproducibility, precision, accuracy, and linearity of this assay. We also compared Nuclisens HIV-1 QT and NASBA HIV-1 QT for the quantitation of HIV-1 RNA levels in clinical specimens and the quantitation of HIV-1 RNA in plasma infected with non-B clade strains of HIV-1.

This study was conducted in two laboratories, one located in Montpellier (laboratory 1) and the other located in Paris (laboratory 2). All of the plasma specimens were independently analyzed in both laboratories, except the specimens of non-B clade HIV-1 strains, which were only analyzed in laboratory 1. For evaluation of specificity and clinical sensitivity, blood was collected on EDTA and immediately centrifuged. One-milliter aliquots of plasma were immediately placed in the lysis buffer provided by the manufacturer, briefly vortexed, and immediately stored at −70°C until testing. For evaluation of reproducibility, precision, accuracy, and linearity, as well as for quantitation of RNA from non-B clades of HIV-1, HIV-1-infected cell culture supernatants were diluted in a pool of plasma samples obtained from HIV-1-seronegative blood donors. Immediately after preparation, these spiked plasma specimens were aliquoted, placed in lysis buffer, and stored as described above. HIV-1 RNA levels were quantitated with Nuclisens HIV-1 QT or NASBA HIV-1 QT in accordance with the manufacturer's instructions.

**Specificity.** Specificity was evaluated on 100 plasma specimens obtained from HIV-1-seronegative blood donors. All of the results were lower than the detection limit in laboratory 1. In laboratory 2, a level of 150 HIV-1 RNA copies/ml (2.17 log HIV-1 RNA copies/ml) was obtained for one specimen. This specimen was tested again, and HIV-1 RNA content was found to be lower than the detection limit. The specificity of Nuclisens HIV-1 QT was therefore 100% in laboratory 1 and 99% in laboratory 2 without repeatability of the initial false-positive result.

**Reproducibility.** Reproducibility was tested on specimens obtained by dilution of a HIV-1 clade B strain (HIV-$1_{GER}$) in a HIV-1-seronegative plasma pool. Three levels of HIV-1 RNA levels were tested. In the high HIV-1 RNA level specimen (level A), the HIV-$1_{GER}$ culture supernatant was diluted to approximately 500,000 HIV-1 RNA copies/ml (5.70 log HIV-1 RNA copies/ml). A low HIV-1 RNA level specimen (level B), which was obtained by diluting the high HIV-1 RNA level specimen 1:200, contained approximately 2,500 HIV-1 RNA copies/ml (3.40 log HIV-1 RNA copies/ml), and a specimen with a very low HIV-1 RNA level (level C) was obtained by 1:10 dilution of the low HIV-1 RNA level specimen (approximately 250 HIV-1 RNA copies/ml; 2.40 log HIV-1 RNA copies/ml). In both laboratories, 10 aliquots of each specimen were tested in one run and the runs were repeated three times on different days. As shown in Table 1, standard deviations

* Corresponding author. Mailing address: Laboratoire de Virologie, Hôpital Saint-Eloi, Centre Hospitalier Universitaire, 34295 Montpellier Cedex 5, France. Phone: (33) 467-337127. Fax: (33) 467-337623. E-mail: msegondy@worldnet.fr.

TABLE 2. HIV-1 RNA levels obtained in the two laboratories by testing spiked plasma specimens containing known quantities of HIV-1 RNA

| Sample no. | Log no. of copies/ml | | |
|---|---|---|---|
| | Input | Laboratory 1 | Laboratory 2 |
| 1 | 6.00 | 5.80 | 5.91 |
| 2 | 6.00 | 5.78 | 5.93 |
| 3 | 6.00 | 5.84 | 6.08 |
| 4 | 5.00 | 4.96 | 4.96 |
| 5 | 5.00 | 4.94 | 4.91 |
| 6 | 5.00 | 4.90 | 5.30 |
| 7 | 4.00 | 4.00 | 4.30 |
| 8 | 4.00 | 3.94 | 4.34 |
| 9 | 4.00 | 4.08 | 3.95 |
| 10 | 3.00 | 3.18 | 3.20 |
| 11 | 3.00 | 2.95 | 2.52 |
| 12 | 3.00 | 2.80 | 3.00 |
| 13 | 2.00 | 2.45 | 2.67 |
| 14 | 2.00 | 1.38 | 1.46 |
| 15 | 2.00 | <LDL[a] | <LDL |

[a] LDL, lower detection limit.

obtained with levels A and B were <0.15 log HIV-RNA copy/ml in the within-run, interrun and interlaboratory reproducibility tests; the coefficients of variation (CV) ranged from 11 to 31% when the results were expressed as HIV-1 RNA copies per milliliter and from 1.0 to 3.9% when the results were expressed as log HIV-1 RNA copies per milliliter. Higher standard deviations (0.21 to 0.31 log copies/ml) and CVs (HIV-1 RNA copies per milliliter, 43 to 103%; log HIV-1 RNA copies per milliliter, 8.2 to 15.4%) were obtained with the level C specimen, indicating a decrease in reproducibility when the HIV-1 RNA levels decreased to values near the lower limit of quantitation.

**Linearity, accuracy, and precision.** Linearity, accuracy, and precision were determined with a panel of spiked plasma samples which contained known quantities of HIV-1 RNA copies (7.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.7, and 1.0 log HIV-1 RNA copies/ml). This panel was obtained by successive dilutions of a HIV-1 suspension in which the number of HIV-1 particles had been determined by electron microscopy (5). Each sample was tested three times by each laboratory. HIV-1 RNA was detected in none of the six samples containing 1.0 log HIV-1 RNA copy/ml, in two of the six samples containing 1.7 log HIV-1 RNA copies/ml (1.86 and 2.18 log HIV-1 RNA copies/ml), and in four of the six samples containing 2.0 log HIV-1 RNA copies/ml (1.38, 1.46, 2.45, and 2.67 log HIV-1 RNA copies/ml). On the other hand, invalid results due to competition between HIV-1 RNA and the Nuclisens HIV-1 QT calibrators were obtained for five of the six samples containing 7.0 log HIV-1 RNA copies/ml; the result was 6.93 log HIV-1 RNA copies/ml in the remaining sample. Results obtained for input HIV-1 RNA concentrations ranging from 2.0 to 6.0 log HIV-1 RNA copies/ml are presented in Table 2. A linear relationship between the input and measured HIV-1 RNA concentrations was observed throughout this range ($r = 0.990$; $R^2 = 0.980$). According to the input HIV-1 RNA concentration, accuracy varied from −0.11 to +0.10 log HIV-1 RNA copy/ml and precision varied from 0.66 to 0.14 log HIV-1 RNA copy/ml. As observed in the reproducibility tests, the precision decreased when the HIV-1 RNA level dropped near the lower limit of quantitation.

**Comparison of Nuclisens HIV-1 QT with NASBA HIV-1 QT for HIV-1 RNA quantitation in clinical samples.** In both lab-

TABLE 1. Within-run, interrun and interlaboratory reproducibility of Nuclisens HIV-1 QT for quantitation of high, low, and very low HIV-1 RNA levels in spiked plasma specimens[a]

| Comparison | Mean HIV-1 RNA level ± SD (CV [%]) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Laboratory 1 | | | | | | Laboratory 2 | | | | | |
| | Specimen A | | Specimen B | | Specimen C | | Specimen A | | Specimen B | | Specimen C | |
| | Copies/ml | Log copies/ml | Copies/ml | Log copies/ml | Copies/ml | Log copies/ml | Copies/ml | Log copies/ml | Copies/ml | Log copies/ml | Copies/ml | Log copies/ml |
| Within run | | | | | | | | | | | | |
| 1 | 430,000 ± 86,281 (20.1) | 5.62 ± 0.09 (1.7) | 2,540 ± 935 (36.8) | 3.38 ± 0.14 (4.3) | 267 ± 276 (103.3) | 2.28 ± 0.35 (15.2) | 451,000 ± 105,034 (23.3) | 5.64 ± 0.09 (1.7) | 2,910 ± 806 (27.7) | 3.45 ± 0.13 (3.9) | 310 ± 132 (42.7) | 2.44 ± 0.23 (9.4) |
| 2 | 521,000 ± 78,095 (15.0) | 5.71 ± 0.06 (1.1) | 2,070 ± 552 (26.7) | 3.30 ± 0.13 (3.8) | 355 ± 318 (89.7) | 2.45 ± 0.29 (11.7) | 410,000 ± 94,163 (23.0) | 5.60 ± 0.10 (1.7) | 2,520 ± 473 (18.8) | 3.39 ± 0.08 (2.3) | 365 ± 203 (55.7) | 2.51 ± 0.21 (8.3) |
| 3 | 424,000 ± 55,817 (13.2) | 5.62 ± 0.05 (1.0) | 2,030 ± 427 (21.0) | 3.30 ± 0.10 (2.9) | 300 ± 194 (64.6) | 2.39 ± 0.29 (12.0) | 579,000 ± 137,877 (23.8) | 5.75 ± 0.13 (2.3) | 2,510 ± 273 (10.9) | 3.40 ± 0.05 (1.3) | 692 ± 464 (67.1) | 2.77 ± 0.23 (8.4) |
| Between II runs | 458,333 ± 84,897 (19.0) | 5.65 ± 0.08 (1.4) | 2,213 ± 691 (31.0) | 3.33 ± 0.13 (3.8) | 307 ± 261 (85.0) | 2.38 ± 0.31 (12.9) | 480,000 ± 132,040 (28.0) | 5.67 ± 0.12 (2.1) | 2,647 ± 575 (22.0) | 3.41 ± 0.09 (2.8) | 456 ± 339 (74.0) | 2.58 ± 0.26 (10.1) |
| Between laboratories | | | | | | | 469,167 ± 110,596 (24.0) | 5.66 ± 0.10 (1.8) | 2,430 ± 667 (27.0) | 3.37 ± 0.12 (3.5) | 381 ± 309 (81.0) | 2.48 ± 0.30 (12.1) |

[a] Specimens: A, high HIV-1 RNA level; B, low HIV-1 RNA level; C, very low HIV-1 RNA level.

TABLE 3. Comparison of NASBA HIV-1 QT and Nuclisens HIV-1 QT for quantitation of HIV-1 RNA from non-B clade strains

| Group and clade | Strain | Log no. of copies/ml | |
|---|---|---|---|
| | | NASBA HIV-1 QT | Nuclisens HIV-1 QT |
| M | | | |
| A | DJ258 | 4.51 | 4.15 |
| | DJ263 | 4.45 | 3.85 |
| C | ZAM18 | 5.00 | 4.36 |
| | ZAM20 | 4.66 | 3.93 |
| D | UG270 | 4.72 | 4.41 |
| | UG274 | 4.52 | 4.11 |
| E | CM235 | 3.98 | 3.46 |
| | CM241 | 4.04 | 3.62 |
| F | 163.3069 | 4.08 | 4.00 |
| | 163.3070 | 3.96 | 3.41 |
| G | G98 | <LDL[a] | <LDL |
| | LBV21 | <LDL | <LDL |
| H | VI557 | 3.59 | 3.08 |
| O[b] | Ant70 | <LDL | <LDL |
| O | MP331 | <LDL | <LDL |
| O | MP450 | <LDL | <LDL |
| O | MP539 | <LDL | <LDL |

[a] LDL, lower detection limit.
[b] HIV-1 RNA and P24 antigen were quantitated in a 1:1,000 dilution of cell culture supernatants. P24 antigen values were 3,220, 6,640, 6690, and 9,040 pg/ml for strains Ant 70, MP331, MP450, and MP539, respectively.

oratories, HIV-1 RNA levels were quantitated by NASBA HIV-1 QT and Nuclisens HIV-1 QT in 78 plasma samples obtained from HIV-1-infected patients. For 53 samples, HIV-1 RNA could be quantitated with NASBA HIV-1 QT with values ranging from 2.61 to 5.76 log HIV-1 RNA copies/ml. A linear relationship was observed between the values obtained with NASBA HIV-1 QT and Nuclisens HIV-1 QT ($r = 0.964$; $R^2 = 0.930$). In 25 samples, HIV-1 RNA was not detected with NASBA HIV-1 QT but could be quantitated with Nuclisens HIV-1 QT in 9 (36%) samples in laboratory 1 (range, 1.96 to 3.20 log HIV-1 RNA copies/ml) and 6 (24%) samples in laboratory 2 (range, 2.04 to 3.00 log HIV-1 RNA copies/ml). The HIV-1 RNA levels measured in both laboratories with Nuclisens HIV-1 correlated well ($r = 0.980$; $R^2 = 0.959$).

**Quantitation of RNA from non-B clade strains of HIV-1.** As shown in Table 3, HIV-1 strains belonging to clade G could be detected by neither NASBA HIV-1 QT nor Nuclisens HIV-1 QT, whereas RNA from strains belonging to the other clades of HIV-1 group M could be quantitated by both assays. Four strains belonging to HIV-1 group O were also tested; RNA could not be detected in the corresponding samples despite detection of HIV-1 antigen (Murex HIV Antigen Mab; Murex Diagnostics, Chatillon, France).

**Conclusion.** Our results indicate that Nuclisens HIV-1 QT is a reliable method for the quantitation of HIV-1 RNA in plasma. Results obtained with Nuclisens HIV-1 QT are closely related to those obtained with NASBA HIV-1 QT, but Nuclisens HIV-1 QT is more suitable than NASBA HIV-1 QT for quantitation of low levels of HIV-1 RNA.

We thank Organon Teknika (Fresnes, France) for providing reagent kits and the electron microscopy-titrated HIV-1 suspension. We thank Martine Peeters (ORSTOM, Montpellier, France) for providing group O and clade G and H HIV-1 strains.

## REFERENCES

1. **Coombs, R. W., S. L. Welles, C. Hooper, P. S. Reichelderfer, R. T. D'Aquila, A. J. Japour, V. A. Johnson, D. R. Kuritzkes, D. D. Richman, S. Kwok, J. Todd, J. B. Jackson, V. DeGruttola, C. S. Crumpacker, and J. Kahn for The AIDS Clinical Trials Group (ACTG) 116B/117 Study Team and The ACTG Virology Committee Resistance and HIV-1 RNA Working Groups.** 1996. Association of plasma human immunodeficiency virus type 1 RNA level with risk of clinical progression in patients with advanced infection. J. Infect. Dis. **174:**704–712.
2. **Coste, J., B. Montès, J. Reynes, M. Peeters, C. Segarra, J. P. Vendrell, E. Delaporte, and M. Segondy.** 1996. Comparative evaluation of three assays for the quantitation of human immunodeficiency virus type 1 RNA in plasma. J. Med. Virol. **50:**293–302.
3. **Katzenstein, D. A., S. M. Hammer, M. D. Hughes, H. Gundacker, J. B. Jackson, S. Fiscus, S. Rasheed, T. Elbeik, R. Reichman, A. Japour, T. C. Merigan, and M. S. Hirsch for The AIDS Clinical Trials Group Study 175 Virology Study Team.** 1996. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic milliliter. N. Engl. J. Med. **335:**1091–1098.
4. **Kern, D., M. Collins, T. Fultz, J. Detmer, S. Hamren, J. J. Peterkin, P. Sheridan, M. Urdea, R. White, T. Yeghiazarian, and J. Todd.** 1996. An enhanced-sensitivity branched-DNA assay for quantification of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. **34:**3196–3202.
5. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, S. R. Conley, J. P. Moore, J. L. Raina, H. Renz, H. R. Gelderblom, and P. L. Nara.** 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology **189:**695–714.
6. **Mellors, J. W., C. R. Rinaldo, P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170.
7. **Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield, and S. Kwok.** 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J. Clin. Microbiol. **32:**292–300.
8. **Revets, H., D. Marissens, S. De Wit, P. Lacor, N. Clumeck, S. Lauwers, and G. Zissis.** 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. J. Clin. Microbiol. **34:**1058–1064.
9. **Schuurman, R., D. Descamps, G. J. Weverling, S. Kaye, J. Tijnagel, I. Williams, R. van Leeuwen, R. Tedder, C. A. B. Boucher, F. Brun-Vézinet, and C. Loveday.** 1996. Multicenter comparison of three commercial methods for quantification of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. **34:**3016–3022.
10. **van Gemen, B., T. Kievits, R. Schukkink, D. van Strijp, L. T. Malek, R. Sooknanan, H. G. Huisman, and P. Lens.** 1993. Quantification of HIV-1 RNA in plasma using NASBA during HIV-1 primary infection. J. Virol. Methods **43:**177–187.
11. **Verhofstede, C., S. Reniers, F. van Wanzeele, and J. Plum.** 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.