

## Multicenter evaluation of the NucliSens EasyQ HIV-1 v1.1 assay for the quantitative detection of HIV-1 RNA in plasma

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### Abstract

The Nuclisens EasyQ HIV-1 v1.1 assay (Biomérieux) is a real-time detection method combined with NASBA technology designed to measure plasma HIV-RNA. Its performance was assessed in 1008 clinical specimens collected from individuals infected with clade B (774) and non-B (234) HIV-1 variants at four European laboratories. The results were compared with those obtained using three other commercial viral load assays: Cobas Amplicor Monitor HIV-1 v1.5 (Roche), Versant HIV-1 RNA assay (Bayer) and Nuclisens HIV-1 QT (Biomérieux). Overall, the linearity, specificity and reproducibility of the EasyQ assay was comparable with that from the other tests. The correlation coefficient ( $R$ ) between methodologies was 0.85 for Amplicor; 0.87 for Versant; and 0.91 for Nuclisens. The specificity of the assay was 99.4%. Of note, Versant missed 17% of specimens with non-B subtypes which could be detected by EasyQ, while Amplicor provided similar results than EasyQ. HIV-1 group O specimens were only detected by the EasyQ assay. In conclusion, the performance of the EasyQ assay seems to be similar to that of other HIV-1 viral load tests currently on the market, but it is more sensitive than Versant for HIV-1 non-B subtypes and shows a wider dynamic range than Amplicor. Moreover, as it incorporates the advantage of real-time detection procedures, it facilitates high throughput and short turnaround time. © 2005 Elsevier B.V. All rights reserved.

**Keywords:** HIV-RNA; Viral load; Real-time PCR; Non-B subtypes

### 1. Introduction

The measurement of human immunodeficiency virus type 1 (HIV-1) RNA in plasma allows accurate prediction of the clinical outcome in a given patient as well as permits assessment of the efficacy of antiretroviral therapies (Connick et al., 2000; Yeni et al., 2004). Due to the increased number of patients and sampling frequency, there is a constant demand for more rapid testing of specimens from individuals infected with HIV. Moreover, since the prevalence of infections due to non-B subtypes is growing rapidly in Europe (Holguín et al., 2003; Parry et al., 2001) and in the United States (Hanna,

2003), there is increasing concern about the quality of patient care, which may be compromised by inaccurate viral load determinations. Therefore there is a growing need for more sensitive, high throughput viral load assays for HIV-infected individuals (Gallant, 2000).

Several methods are commercially available for the quantitative detection of HIV-1 RNA in plasma (Murphy et al., 2000). These assays use technologies based upon either signal amplification (e.g., the Versant HIV-1 RNA assay) or target amplification, such as the Amplicor HIV-1 Monitor and the NucliSens HIV-1 QT assays. The performance characteristics of an assay, including its dynamic range, lower limit of detection, accuracy, specificity, reproducibility and the ability to detect and accurately quantify different HIV-1 subtypes are key factors for the selection of a given test.

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In this study, the performance of the NucliSens EasyQ HIV-1 assay, the first commercially available real-time nucleic acid-based test for HIV-1 RNA measurement in routine diagnostic laboratories, was described. The assay uses the NASBA amplification technology followed by real-time detection with molecular beacons (Tyagi and Kramer, 1996; Van Beuningen, 2001; Weusten et al., 2002).

## 2. Materials and methods

### 2.1. Sites

The evaluation of the NucliSens EasyQ HIV-1 assay (EasyQ assay) was performed at the following four different laboratories: Sanquin, Division CLB, Amsterdam, The Netherlands (site 1), CHU St Eloi, Montpellier, France (site 2), CHU Hôtel Dieu, Nantes, France (site 3) and Hospital Carlos III, Madrid, Spain (site 4).

### 2.2. Panels and controls

Negative control samples (pooled human plasma) and positive controls containing 15,000, 1500 and 300 copies of HIV-1 RNA (HXB2) were prepared by BioMérieux and provided to the evaluating sites. Control samples were included in each run of the assay.

### 2.3. Patient samples

A total of 774 randomly selected EDTA plasma samples were collected from HIV-1 infected patients. Samples were frozen at  $-80^{\circ}\text{C}$  until their use. One milliliter was tested with the EasyQ HIV-1 assay. In addition, each sample was tested with the assay generally used for routine HIV-1 viral load measurements at each laboratory.

### 2.4. Subtype samples

Panels 301, 302 and 201 containing different HIV-1 subtypes/groups were obtained from Boston Biomedica Incorporation (BBI, West Bridgewater, MA, USA), Walter Reed Army Institute of Research (WRAIR, Rockville, MD, USA) and from NIBSC (Potters Bar, UK). Information about each panel is available at the web site for each institution. Each panel consist of samples representing Group M, subtypes A–K, and a negative control (panels BBI PRD201, PRD302; WRAIR and NIBSC) and samples from HIV-1 group O (panel BBI PRD301). In addition, a total of 234 clinical samples for which the subtype/group already was known were obtained from the sites participating in the study.

### 2.5. Viral load assays

Nucleic acid extractions (for EasyQ HIV-1) from 1 or 2 ml of plasma were performed using the NucliSens Extractor

(BioMérieux, Boxtel, The Netherlands). For the other assays, the nucleic acid was isolated according to the manufacturer's instructions.

Three different viral load tests were used as reference to compare the results obtained with EasyQ HIV-1 (BioMérieux), whose detailed principles have been described elsewhere (Van Beuningen, 2001; Weusten et al., 2002). These assays were: (1) VERSANT<sup>®</sup> HIV-1 RNA v3.0 (Bayer), which requires 1 ml of plasma. The UQL and LLQ are 500,000 copies/ml (5.7 log) and 50 copies/ml (1.7 log), respectively (Erice et al., 2000); (2) COBAS AMPLICOR<sup>™</sup> HIV-1 MONITOR v1.5 (Roche), which requires 0.5 ml of plasma. The UQL and LLQ are 75,000 copies/ml (4.88 log) and 50 copies/ml (1.7 log), respectively (Sun et al., 1998); and (3) Nuclisens HIV QT (Biomerieux), with has a dynamic range from 50 copies/ml (1.7 log) to 3,000,000 copies/ml (6.48 log) and uses 1 ml of plasma (Ginocchio et al., 2003).

For the Nuclisens EasyQ assay, primers and probes are designed based on well-conserved *gag* region of HIV-1. During the amplification process there is a constant growth in the concentration of amplicons to which the beacon can bind while generating a fluorescence signal. The overall fluorescence curve contains kinetic information on both, amplicon formation and beacon binding. The quantitation is based on assessing the amplicon formation rate from the viral RNA relative to that from a fixed amount of calibrator RNA. The linear dynamic range of the assay runs from 50 IU/ml (1.7 log) to 3,000,000 IU/ml (6.48 log) (Weusten et al., 2002). Results were expressed as International Units per ml (IU/ml) for the EasyQ whereas it was HIV-RNA copies/ml for the rest of the assays.

### 2.6. Specificity, linearity and sensitivity

The specificity of the EasyQ HIV-1 assay was assessed by testing 441 plasma specimens from a low-risk blood donor population (all donor samples were found to be non-reactive for antibodies to HIV-1 nor HIV-2 using an FDA licensed assay (AxSYM, Abbott Laboratories, Chicago, IL).

The linearity and sensitivity of the assay were assessed on dilution series of an HIV-1 RNA control (subtype B). This secondary standard was diluted in HIV-negative plasma, resulting in the following concentrations: 25, 50, 100, 200, 400, 1000, 3000, 10,000, 30,000, 100,000, 300,000, 1,000,000 and 3,000,000 IU/ml. Inputs were tested 12-fold at each site. To assess the effect of sample input volume on linearity and sensitivity, 1 and 2 ml of plasma were used for nucleic acid extraction.

### 2.7. Statistical analyses

Data were  $\log_{10}$  transformed prior to be analysed. Inter-run, intra-run and total assay variations were derived from the respective averages and standard deviations. Assay linearity for Nuclisens EasyQ was assessed graphically by parallel line analysis related to dilutions.

The 50% and 95% detection limits were estimated by probit analysis using SPSS version 9.0. Paired sample *t*-test statistics was used to compare the results obtained using two different assays. For concordance analyses between two methods a linear regression analyses and the Bland and Altman approach (Bland and Altman, 1986) were used.

### 3. Results

#### 3.1. Specificity, sensitivity and dynamic range

The specificity of the NucliSens EasyQ HIV-1 assay testing 441 HIV-negative plasma specimens collected from blood donors was 99.3%. There were three initially positive samples and only one was found to be repeatedly positive, with values always below 200 IU/ml.

The percentage-hit rate was determined at each input level. Using probit analyses, the calculated 50% and 95% detection limits using 1 ml sample input were 62 and 357 IU/ml, respectively. For 2 ml sample input, the detection limits were 36 and 141 IU/ml, respectively. Using 1 ml, the 50% and 95% detection rates for the HIV-1 QT assay, were 71 and 331 IU/ml, respectively.

The EasyQ assay has a linear range from 50 to  $3 \times 10^6$  IU/ml. Precision, defined as the standard deviation of the log-transformed values, was approximately 0.17 log units at concentrations above 1000 IU/ml, and 0.26 log units for values lower than 1000 IU/ml.

Quantitative results obtained by different lots and sites were similar, showing a good inter-lot and inter-site reproducibility of the EasyQ assay (see Fig. 1).

#### 3.2. Correlation between EasyQ HIV-1 and other assays

Table 1 summarizes the concordance agreement between EasyQ and other viral load assays, including the Nuclisens HIV-1 QT (Biomerieux) (HIV-1 QT), branched DNA (Versant HIV-1 RNA v3.0; Bayer), and Cobas Amplicor HIV-1 Monitor v1.5 (Roche); (Amplicor). Concordant results comparing EasyQ and HIV-1 QT were obtained in 255/268 samples (95%), and showed a good correlation ( $y = 0.8271x + 0.5109$ ,  $R^2 = 0.83$ ) (Fig. 2A). Concordant results were obtained in 276/309 (89%) of samples comparing EasyQ assay and Amplicor ( $y = 0.817x + 0.2585$ ,  $R^2 = 0.75$ ). Overall, the EasyQ provided lower values than the Amplicor

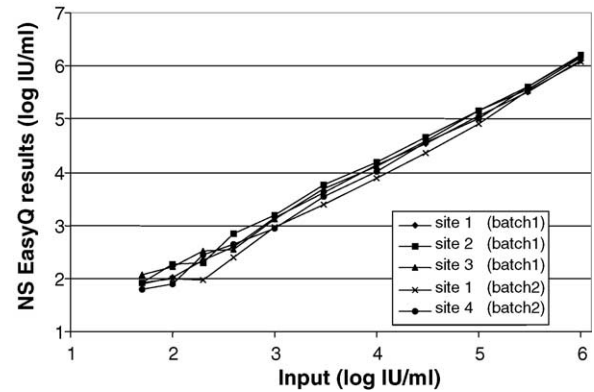


Fig. 1. Linear dynamic range obtained at the four laboratory sites.

(Fig. 2B). Most probably this is due to the fact that results obtained with Amplicor are given as copies/ml. EasyQ reports in IU/ml.

For EasyQ and Versant, a total of 176 (89%) concordant results were recorded ( $y = 0.8704x + 0.47$ ,  $R^2 = 0.72$ ) (Fig. 2C). Most specimens yielding a positive result with either one of these assays were found to be in the lower limit of the quantitative range of these tests.

No significant differences between EasyQ and HIV-1 QT or Versant were observed between IU/ml versus copies/ml, whereas for the Amplicor a conversion factor of 0.51 should be used to calculate IU/ml from copies/ml.

#### 3.3. HIV-1 subtype detection and quantitation

The results testing panels with non-B subtypes are shown in Fig. 3. All isolates from HIV-1 group M subtypes A–H were detected by the EasyQ assay. Overall, the results obtained were similar to those obtained using the other three reference assays, although the Amplicor yielded the highest values.

The performance of the different viral load assays on distinct HIV-1 subtypes was further investigated by testing clinical samples identified as group M subtypes A–H and J, and HIV-1 group O from patients in regular follow-up at the four clinical sites. A large proportion of these specimens had been collected from subjects receiving antiretroviral therapy. The EasyQ results were compared in parallel with those obtained using Amplicor or Versant at sites 2 and 4, respectively (Table 2). The total number of samples detected by EasyQ resulted to be similar to that recognised using Amplicor. In

Table 1

Concordance between the results using Nuclisens EasyQ HIV-1 assay and other commercial viral load assays

	HIV-1QT (N=268)		Versant (N=197)		Amplicor (N=309)	
	Positive	Negative	Positive	Negative	Positive	Negative
EasyQ						
Positive	138	11	141	12	185	11
Negative	2	117	9	35	22	91

EasyQ, Nuclisens EasyQ (Biomerieux); HIV-1 QT, Nuclisens HIV-1 QT (Biomerieux); Versant, branched DNA (Versant HIV-1 RNA v3.0; Bayer); Amplicor, Cobas Amplicor HIV-1 Monitor v1.5 (Roche).

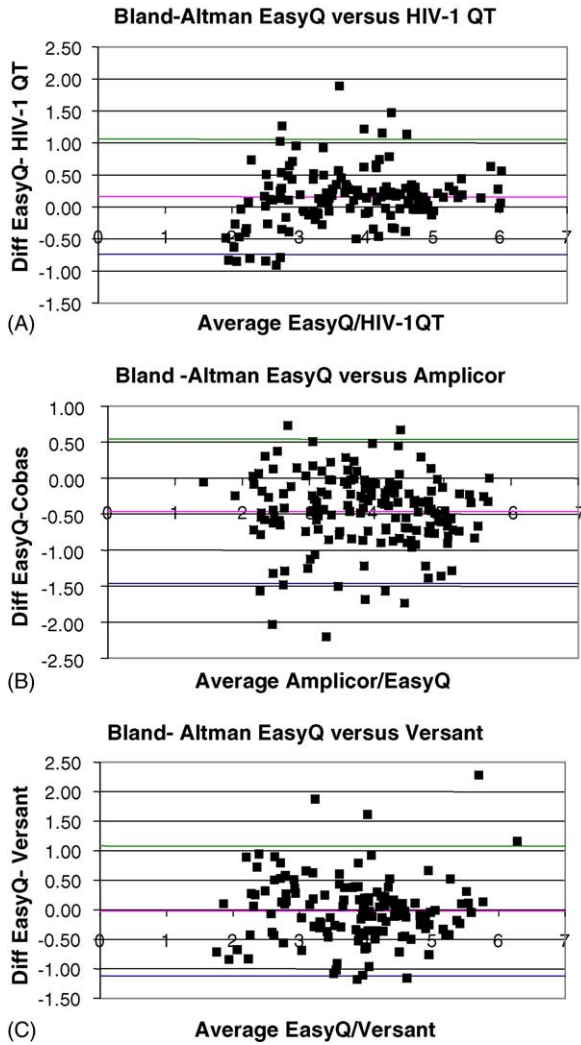


Fig. 2. Differences between viral load values obtained comparing EasyQ with Amplicor (A), Versant (B) and HIV-1QT (C). The mean difference (purple line) and plus and minus two standard deviations are shown (green and blue lines). Values were expressed as log HIV-RNA copies/ml for Versant, HIV-QT and Amplicor and as log HIV-RNA IU/ml for EasyQ.

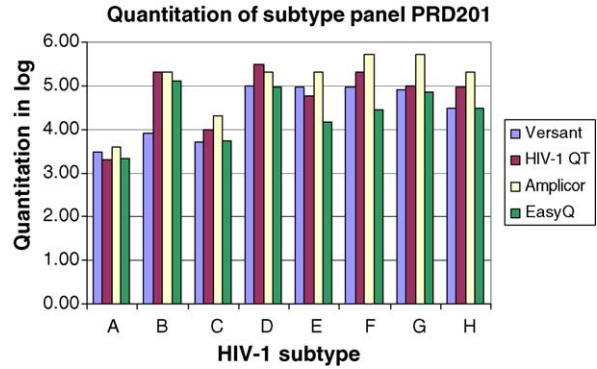


Fig. 3. Quantitative results testing a reference panel PRD201 (BBI) of non-B subtypes using different HIV-1 viral load assays.

contrast, Versant detected a significantly lower number of these samples than the EasyQ; up to 17% of samples measured by EasyQ were missed by Versant.

Finally, HIV-1 group O specimens were detected by EasyQ and were missed by Amplicor and Versant detected a lower proportion of them. Quantitative values should be compared with an optimized quantitative assay.

#### 4. Discussion

Viral load assays are widely used in the management of HIV-infected patients, and important decisions are taken about initiation or change in antiretroviral therapy based on HIV-RNA copy numbers (Pozniak et al., 2003; Yeni et al., 2004). The suppression of plasma HIV-RNA to undetectable levels (below 50 copies/ml) is required for preventing viral rebound and development of drug resistance in subjects under antiretroviral therapy (Havlir et al., 2000; Raboud et al., 1998). The availability of viral load tests with a reliable and sensitive limit of detection is crucial for this purpose.

The analytical and clinical performance of the EasyQ HIV-1 v1.1 assay was evaluated at four different laboratories. The

Table 2  
 Comparison of the proportion of samples detected by EasyQ, Amplicor and Versant at sites 2 and 4

HIV subtype	Site 2		Site 4			
	No. of tested	Amplicor (%)	EasyQ (%)	No. of tested	Versant (%)	EasyQ (%)
A	28	28 (100)	26 (93)	14	7 (50)	12 (86)
B	47	47 (100)	44 (94)	17	11 (65)	17 (100)
C	7	6 (86)	5 (71)	11	4 (36)	6 (54)
D	4	4 (100)	4 (100)	2	1 (50)	2 (100)
E	8	8 (100)	7 (88)	–	–	–
F	3	3 (100)	3 (100)	2	2 (100)	1 (50)
G	15	15 (100)	15 (100)	46	25 (54)	26 (58)
H	1	1 (100)	1 (100)	2	1 (50)	1 (50)
J	2	2 (100)	2 (100)	2	2 (100)	2 (100)
A/C	–	–	–	1	0	0
F/B	–	–	–	3	1 (33)	1 (33)
HIV-1 group O	6	0	3 (50)	13	4 (31)	9 (69)
Total	121	114 (94)	110 (91)	113	58 (51)	77 (68)

total number of tests performed during this multicentre evaluation was large, of approximately 4000, which represented over 140 test runs. The overall percentage of initial invalid results was 2% (data not shown). Upon repeated amplification and detection of these invalid samples, the majority of them provided a valid result that was used for further calculations.

The performance of the EasyQ in terms of sensitivity, linearity, reproducibility and specificity was comparable to that obtained using other viral load assays currently used in clinical practice (Brambilla et al., 2001; Murphy et al., 2000; Nolte et al., 1998; Vandamme et al., 1996). The sensitivity was similar to that of the HIV-1 QT assay, using either 50% or 95% detection limits. The 95% detection limit obtained with EasyQ HIV-1 was in line with the 95% detection limit previously reported for the Amplicor assay (Sun et al., 1998), and was better than that reported for the Versant assay (205 copies/ml) (Erice et al., 2000).

In respect to the linear dynamic range, the EasyQ showed a dynamic range between 50 and 3,000,000 IU/ml, which is much broader than that for any other viral load assay (Dyer et al., 1999; Ginocchio et al., 2003). This is particularly relevant in respect to the Amplicor v1.5, which has a dynamic range ending at 75,000 copies/ml (Sun et al., 1998). The clinical implication of these findings may be particularly significant for assessing the initial viral response after initiating antiretroviral therapy, following international guidelines (Pozniak et al., 2003; Yeni et al., 2004).

The specificity of the EasyQ approached 100%. False positive results have been reported using other viral load tests (De Mendoza et al., 1998; Havlicek and Hage-Korban, 1999), which has supported the recommendation to avoid their use for diagnostic purposes (De Mendoza et al., 1998).

The original nucleic acid-based tests were developed based primarily on sequence information derived from HIV-1 subtype B, the most frequent variant circulating in the United States and Western Europe. However, the co-circulation of distinct HIV-1 subtypes is growing rapidly (Hanna et al., 2003; Holguín et al., 2003; Parry et al., 2001), and therefore much concern exists on the deleterious influence of genetic diversity on the efficiency of currently available viral load assays. Failures to detect or underestimate HIV-RNA in specimens from people infected with non-B variants have been reported (Alaeus et al., 1997; Jenny-Avital and Beatrice, 2001; Loussert-Ajaka et al., 1995; Triques et al., 1999). In our study, the results obtained using EasyQ and Amplicor by testing well-established subtype panels and clinical samples collected from subjects infected with non-B subtypes were compared. Overall, EasyQ demonstrated similar performance than the Amplicor, but was superior to Versant. In respect to HIV-1 group O, the EasyQ detected a higher number of samples. However, a more detailed analysis comparing quantitative results with an optimized assay is needed.

The use of the real-time technology in the EasyQ permits a strong increase in the throughput compared to the end-point detection used by the currently commercial viral load tests. Amplification and real-time detection of 48 samples

took only 90 min with approximately 30 min hands-on time. This aspect may be particularly attractive for diagnostic laboratories, in which many samples need to be processed in a short period of time.

In summary, the performance of the EasyQ HIV-1 assay seems to be similar to that of other HIV-1 viral load assays currently on the market, although the assay shows a better performance than Versant when testing HIV-1 non-B subtypes and a wider dynamic range than Amplicor. Moreover, since the technique is fully internally calibrated and based upon real-time amplification and detection, that permits a high throughput, and a short turnaround time, which may represents a significant advantage for clinical laboratories.

## References

- Alaeus, A., Lidman, K., Sonnenborg, A., Albert, J., 1997. Subtype specific problems with quantification of plasma HIV-1 RNA. *AIDS* 11, 859–865.
- Bland, J., Altman, D., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1, 307–310.
- Brambilla, D.J., Granger, S., Jennings, C., Bremer, J.W., 2001. Multisite comparison of reproducibility and recovery from the standard and ultrasensitive Roche Amplicor HIV-1 Monitor assays. *J. Clin. Microbiol.* 39, 1121–1123.
- Connick, E., Lederman, M., Kotzin, B., Spritzler, J., Kuritzkes, D., St Clair, M., Sevin, A., Fox, L., Chiozzi, M., Leonard, J., Rousseau, F., D'Arc Roe, J., Martinez, A., Kessler, H., Landay, A., 2000. Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response. *J. Infect. Dis.* 181, 358–363.
- De Mendoza, C., Holguin, A., Soriano, V., 1998. False positives for HIV using commercial viral load quantification assays. *AIDS* 12, 2076–2077.
- Dyer, J.R., Pilcher, C.D., Shepard, R., Schock, J., Eron, J.J., Fiscus, S.A., 1999. *J. Clin. Microbiol.* Comparison of NucliSens and Roche Monitor assays for quantitation of levels of HIV type 1 RNA in plasma 37, 447–449.
- Erice, A., Brambilla, D., Bremer, J., Jackson, J.B., Kokka, R., Yen-Lieberman, B., Coombs, R., 2000. Performance characteristics of the Quantiplex HIV-1 RNA 3.0 assay for detection and quantitation of HIV type 1 RNA in plasma. *J. Clin. Microbiol.* 38, 2837–2845.
- Gallant, J.E., 2000. Strategies for long-term success in the treatment of HIV infection. *JAMA* 283, 1329–1334.
- Ginocchio, C., Kemper, M., Stellrecht, K.A., Witt, D., 2003. Multicenter evaluation of the performance characteristics of the Nuclisens HIV-1 QT assay used for quantitation of HIV-1 RNA. *J. Clin. Microbiol.* 41, 164–173.
- Hanna, G.J., Balaguera, H.U., Freedberg, K.A., Werner, B.G., Steger Crav, K.A., Craven, D.E., D'Aquila, R., 2003. Drug-selected mutations and non-B subtypes in antiretroviral-naïve adults with established HIV infection. *J. Infect. Dis.* 188, 986–991.
- Havlicek, D.H., Hage-Korban, E., 1999. False-positive HIV diagnosis by HIV-1 plasma viral load testing. *Ann. Int. Med.* 131, 37–39.
- Havliir, D.V., Hellmann, N.S., Petropoulos, C.J., Whitcomb, J.M., Collier, A.C., Hirsch, M.S., Tebas, P., Sommadossi, J.P., Richman, D.D., 2000. Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens. *JAMA* 283, 229–233.
- Holguín, A., Alvarez, A., Pena, M.J., Artiles, F., Molina, L., Soriano, V., 2003. HIV-positive immigrants in the Canary Islands Spain: implications for public health in Europe. *HIV Clin. Trials* 4, 184–192.
- Jenny-Avital, E., Beatrice, S., 2001. Erroneously low or undetectable plasma HIV-1 RNA load, determined by PCR, in West African and

- American patients with non-B subtype HIV-1 infection. *Clin. Infect. Dis.* 32, 1227–1230.
- Loussert-Ajaka, I., Descamps, D., Simon, F., Brun-Vézinet, F., Ekwalanga, M., Sarogosti, S., 1995. Genetic diversity and HIV detection by polymerase chain reaction. *Lancet* 346, 912–913.
- Murphy, D.G., Côté, L., Fauvel, M., René, P., Vincelette, J., 2000. Multicenter comparison of Roche Cobas Amplicor Monitor version 1.5, Organon-Teknika Nuclisens QT with extractor, and Bayer Quantiplex version 3.0 for quantification of HIV type 1 RNA in plasma. *J. Clin. Microbiol.* 38, 4034–4041.
- Nolte, F.S., Boysza, J., Thurmond, C., Clark, W.S., Lennox, J.L., 1998. Clinical comparison of an enhanced-sensitivity branched-DNA assay and reverse transcription-PCR for quantitation of HIV type 1 RNA in plasma. *J. Clin. Microbiol.* 36, 716–720.
- Parry, J.V., Murphy, G., Barlow, K., Lewis, K., Rogers, P., Belda, F., Nicoll, A., McGarrigle, C., Cliffe, S., Mortimer, P., Clewley, J., 2001. National surveillance of HIV-1 subtypes for England and Wales. *J. Acquir. Immun. Def. Syndr.* 26, 381–388.
- Pozniak, A., Gazzard, B., Anderson, J., Babiker, A., Churchill, D., Collins, M., Fisher, M., Johnson, M., Khoo, S., Leen, C., Loveday, C., Moyle, G., Nelson, M., Peter, B., Phillips, A., Pillay, D., Wilkins, E., Youle, M., 2003. BHIVA Writing Committee BHIVA Executive Committee. British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy. *HIV Med.* 4, 1–41.
- Raboud, J.M., Montaner, J.S., Conway, B., Rae, S., Reiss, P., Vella, S., Cooper, D., Lange, J., Harris, M., Wainberg, M.A., Robinson, P., Myers, M., Hall, D., 1998. Suppression of plasma viral load below 20 copies/ml is required to achieve a long-term response to therapy. *AIDS* 12, 1619–1624.
- Sun, R., Ku, J., Jayakar, H., Kuo, J.C., Brambilla, D., Herman, S., Rosenstrauss, M., Spadaro, S., 1998. Ultrasensitive reverse transcription-PCR assay for quantitation of HIV type 1 RNA in plasma. *J. Clin. Microbiol.* 36, 2964–2969.
- Triques, K., Coste, J., Perret, J.L., Segarra, C., Mpoudi, E., Reynes, J., Delaporte, E., Butcher, A., Dreyer, K., Herman, S., Spadaro, J., Peeters, M., 1999. Efficiencies of four versions of the AMPLICOR HIV-1 MONITOR test for quantification of different subtypes of HIV type 1. *J. Clin. Microbiol.* 37, 110–116.
- Tyagi, S., Kramer, F.R., 1996. Molecular beacons: probes that fluoresce upon hybridisation. *Nat. Biotechnol.* 14, 303–308.
- Van Beuningen, R., 2001. Development of a high throughput detection system for HIV-1 using real-time NASBA based on molecular beacons. In: Raghavachari, R., Tan, W. (Eds.), *Genomics and Proteonomics Technologies, Proceedings of the SPIE. Society of Photo-optical Instrumentation Engineers, Washington, DC*, pp. 66–72.
- Vandamme, A.M., Schmit, J.S., Van Dooren, S., Van Laethem, K., Gobbers, E., Kok, W., Goubau, P., Witvrouw, M., Peetermans, W., De Clerq, E., Desmyter, J., 1996. Quantification of HIV-1 RNA in plasma: comparable results with the NASBA HIV-1 RNA QT and the AMPLICOR HIV Monitor test. *J. Acquir. Immun. Def. Syndr.* 13, 127–139.
- Weusten, J.J., Carpay, W.M., Oosterlaken, T., van Zuijlen, M., Van de Wiel, P., 2002. Principles of quantitation of viral loads using nucleic acid sequence-based amplification in combination with homogeneous detection using molecular beacons. *Nucl. Acids Res.* 30, e26.
- Yeni, P., Hammer, S., Hirsch, M., Saag, M., Schechter, M., Carpenter, C., Fischl, M., Gatell, J., Gazzard, B., Jacobsen, D., Katzenstein, D., Montaner, J., Richman, D., Schooley, R., Thompson, M., Vella, S., Volberding, P., 2004. Treatment for adult HIV infection 2004 Recommendations of the International AIDS Society-USA Panel. *JAMA* 292, 251–265.