J Lab Med 2005;29(1):44–49 © 2005 by Walter de Gruyter • Berlin • New York. DOI 10.1515/JLM.2005.007

Molecular detection of human immunodeficiency virus: where do we stand?

Molekularer Nachweis des humanen Immunschwächevirus: Aktueller Stand

Harald H. Kessler*

Molecular Diagnostics Laboratory, Institute of Hygiene, Medical University of Graz, Graz, Austria

Abstract

Infection with the human immunodeficiency virus (HIV) continues to present diagnostic and therapeutic challenges worldwide. Today, many diagnostic laboratories have implemented assays based on molecular techniques for the detection of HIV. Detection of primary HIV infection, however, still relies on serologic testing, except for the screening of blood and plasma donors, which is done by the use of molecular assays. Additionally, molecular methods are employed for the detection of vertical transmission and monitoring of patients undergoing antiretroviral therapy. Although a continuous improvement in assay performance has been observed during recent years, the lack of comparability of different molecular assays remains a problem. Moreover, a better standardization of molecular assays for the detection of HIV-1 group O and HIV-2 must be addressed in the future.

Keywords: automation; donor screening; human immunodeficiency virus; molecular diagnostics; standardization; therapy monitoring; window period.

Zusammenfassung

Die Infektion mit dem humanen Immunschwächevirus (HIV) stellt nach wie vor weltweit eine diagnostische und therapeutische Herausforderung dar. Heutzutage haben viele diagnostische Laboratorien molekulare Tests zum Nachweis von HIV eingeführt. Die Erstdiagnose der HIV-Infektion beruht jedoch weiterhin auf der Serologie. Davon ausgenommen ist das Screening von Blut- und Plasmaspendern, das mittels molekularer Tests durchgeführt wird. Darüber hinaus werden molekulare Methoden zum Nachweis der vertikalen Übertragung und zum Monitoring von Patienten unter antiretroviraler Therapie eingesetzt. Obwohl während der letzten Jahre eine ständige Verbesserung der Testperformance zu beobachten war, bleibt die mangelnde Vergleichbarkeit verschiedener molekularer Tests ein Problem. Darüber hinaus muss in Zukunft die Standardisierung molekularer Tests zum Nachweis der HIV-1-Gruppe O und von HIV-2 verbessert werden.

Schlüsselwörter: Automatisierung; diagnostisches Fenster; humanes Immunschwächevirus; molekulare Diagnostik; Spenderscreening; Standardisierung; Therapiemonitoring.

Introduction

The human immunodeficiency virus (HIV) is recognized as the etiological agent of acquired immune deficiency syndrome (AIDS). AIDS has become one of the most important global illnesses. The UNAIDS (www.unaids.org) global summary, published at the end of 2003, estimates that 34 to 46 million people are living with HIV/AIDS. In 2003, 4.2 to 5.8 million people were newly infected and 2.5 to 3.5 million people died of AIDS. The virus is mainly transmitted by sexual contact, transfusion of contaminated blood (e.g., intravenous drug abuse), and vertical transmission. Two different HIV types have been identified, HIV-1 and HIV-2. In addition, three groups within the HIV-1 family have been isolated and designated as group M (major), group N (near M), and group O (outlier). Group M can be further divided into several subgroups responsible for the majority of HIV infections worldwide. One of the major steps in restricting the spread of the disease is to inform people about their HIV status. In the industrial world, about 30% do not know they are infected with the virus, whereas this figure reaches almost 90% in the developing world.

Molecular assays

Diagnostic assays based on molecular techniques are recognized as the method of choice for the identification of a large and still growing number of pathogens. Many

*Correspondence: Harald H. Kessler, Molecular Diagnostics Laboratory, Institute of Hygiene, Medical University of Graz, Universitaetsplatz 4, 8010 Graz, Austria
E-mail: harald.kessler@meduni-graz.at

clinical and public health diagnostic laboratories have implemented molecular techniques because they are faster and more accurate than traditional methods. Molecular methods may have a significant impact on detection, therapy monitoring, and resistance testing of HIV. It is, therefore, of major importance for the routine diagnostic laboratory to report accurate and reliable results.

To meet the requirements of quality assurance and quality control, it is essential for the laboratory to participate in an external quality assessment program. Based on data of the Quality Control for Molecular Diagnostics (QCMD; www.qcmd.org) 2004 Human Immunodeficiency Virus Proficiency Program for detection of HIV-1 RNA in plasma, the majority of participating laboratories use commercially available tests for the detection of HIV RNA (Table 1). Compared to in-house assays, these tests showed a significantly better performance. In-house assays are usually labor-intensive and time-consuming. They include a large number of manual steps and are prone to human error. It is therefore strongly recommended to use more automated and standardized assays for the detection of HIV in the routine diagnostic laboratory. A comparative summary of the commercial assays most frequently used in Europe is provided in Table 2. Attention must still be paid to technical limitations, even when commercially available assays are employed. Sample preparation is currently considered the major weakness in molecular assays for detection of HIV RNA. Conventional sample preparation protocols are usually time-consuming, labor-intensive, and susceptible to contamination. It has been demonstrated that the probability of false-positive results due to contamination increases in relation to the number of manipulations involved in sample processing [1, 2]. To save time and labor, more rapid nucleic acid extraction protocols with fewer manipulation steps have largely replaced conventional protocols. Several ready-to-use sample preparation kits, available either separately or as a part of entire molecular kits, were brought on the market and found suitable for inclusion in molecular assays for detection of RNA viruses [4–6]. Recently, automated specimen preparation instruments have been used for automated extraction of HIV-1 RNA [7–10].

For commercially available tests, several comparative studies have been published [11–14]. The results obtained by the majority of these studies did not show significant differences when comparing different assays. Problems may arise because these assays have usually been optimized for the detection of subtype B of HIV-1 [15]. They may still detect and accurately measure viral loads for all group M subtypes [16, 17]. However, it has been reported that some commercially available assays either underevaluate or fail to detect HIV-1 RNA subtypes A, E, and G and other minor strains [18–21]. Additionally, suboptimal performance of assays in patients infected with circulating recombinant forms has been described [22, 23]. The QCMD 2004 HIV Proficiency Panel included HIV-1 subtypes B and G.

Table 1 Assay types employed with the QCMD 2004 HIV Proficiency Panel by participating laboratories providing a total of 113 data sets (modified from [3]).

Assay types (manufacturer)	Number (%)
RT-PCR (Roche)	75 (66.4)
bDNA (Bayer)	14 (12.4)
NASBA (bioMérieux)	7 (6.2)
RT-PCR (Abbott)	5 (4.4)
Other	3 (2.6)
Total commercial	104 (92.0)
In-house real-time	5 (4.4)
In-house conventional	4 (3.6)
Total in-house	9 (8.0)

Detection of primary HIV infection

The standard algorithm for detection of primary HIV infection still relies primarily on serology. The use of enzyme-linked immunosorbent assays (ELISA) is the most rational and cost-effective approach. ELISA is thus widely used to screen human serum and plasma for the presence of anti-HIV antibodies. Because false-positive reactions have frequently been observed, repeatedly reactive samples must be confirmed by other reliable techniques. The confirmatory assay must be able to detect all groups of HIV-1 as well as HIV-2. This is, however, currently impossible when employing a single molecular assay. Therefore, assays based on the western blot principle are still the method of choice for confirmation of anti-HIV antibodies.

The major disadvantage of assays based on detection of anti-HIV antibodies is the so-called “serologic window period”. The appearance of anti-HIV antibodies requires on the average 22 days for the HIV-infected to develop an immune response (Table 3). During the serologic window period, the patient is infected (and infectious), but tests on anti-HIV antibodies are still negative. In order to reduce the serologic window period, assays for detection of the HIV antigen (p24 antigen) have been developed. The p24 antigen requires 16 days on the average for a measurable level to develop (Table 3). Furthermore, antigenemia declines approximately 14 days later and a second window period may be observed when the level of anti-HIV antibodies is still too low. To overcome these problems, assays based on the ELISA technique which permit the simultaneous detection of HIV antigen and anti-HIV antibodies have been developed and shown to be useful [24–26]. By introduction of nucleic acid-amplification tests (NAT), the diagnostic window period can be reduced to approximately 11 days (Table 3). This is of particular interest for detection of HIV infection in transplant donors, in case of needlestick injury, in rape victims, and generally in transfusion medicine. In the context of screening blood and plasma donors, high-sensitive qualitative NAT have been developed based on RT-PCR and transcription-mediated amplification (TMA) technologies. To increase capacity and reduce cost, nucleic acid test assays are performed in the donor

Table 2 Comparison of the most frequently used commercial assays in Europe. Characteristics according to information of manufacturers.

Characteristic	Manufacturer and information						
	Abbott	Bayer HealthCare	bioMérieux	Roche Molecular Diagnostics			
Kit name	LCx HIV Quantitative	VERSANT HIV-1 RNA 3.0	NucliSens HIV-QT	COBAS AMPLICOR HIV-1 MONITOR 1.5	COBAS AmpliPrep AMPLICOR HIV-1 MONITOR	COBAS AmpliScreen HIV-1 1.5	COBAS TAQMAN HIV-1
Sample size (μL)	200 or 1000	1000	1000	200 or 500	700	500	500
Sample preparation	Manual	Manual	Automated	Manual	Automated	Manual	Manual
Amplification method	RT-PCR (target)	bDNA (signal)	NASBA (target)	RT-PCR (target)	RT-PCR (target)	RT-PCR (target)	RT-real time PCR (target)
Detection method	Microparticle EIA	Chemiluminescence	Electrochemiluminescence	EIA	EIA	EIA	Fluorescence
Standards and controls	1 internal QS, 6 external calibrators, 3 external controls	6 external standards, 3 external controls	3 internal calibrators	1 internal QS, 3 external controls	1 internal QS, 3 external controls	1 internal control, 2 external controls	1 internal QS, 3 external controls
Range of linearity (copies/mL)	178–5.0×10^6 (200 μL) 50–1.0×10^6 (1.000 μL)	50–5.0×10^5	51–5.4×10^6	400–7.5×10^5 (200 μL) 50–1.0×10^5 (500 μL)	50–7.5×10^4	Qualitative (lower limit of 600 in a pool with 23 uninfected units)	40–1.0×10^7

Abbreviations: RT-PCR, reverse transcriptase PCR; NASBA, nucleic acid sequence-based amplification; EIA, enzyme linked immunoassay; QS, quantitation standard.

Table 3 Window periods for different detection methods.

Window	Days (average)
Infection to antibody	22
Infection to p24 antigen	16
Infection to HIV RNA	11

screening setting on minipools of 16 to 24 samples. It has been shown that NAT screening is able to reduce the residual risk of transfusion-associated infection for HIV-1 by shortening the diagnostic window period and is superior to p24 antigen testing [27–30]. There remains, however, a residual risk, as the virus load may be below the limit of detection of minipool testing [31]. Individual testing of each sample may overcome this problem, but produces significant costs. Furthermore, NAT appears to be useful for exclusion or confirmation of vertical transmission and for testing immunocompromised patients with poor or even a lack of anti-HIV antibody production.

Monitoring of anti-HIV therapy

As high HIV-1 RNA plasma levels are associated with a more rapid disease progression and an increased risk for vertical transmission, the determination of HIV-1 RNA plasma levels is essential for evaluation of the response to antiretroviral therapy [32–33, 3]. Quantitation of HIV-1 RNA in plasma may be achieved by different types of molecular assays including RT-PCR, bDNA, and nucleic acid sequence-based amplification (NASBA) techniques. In Europe, the COBAS AMPLICOR HIV-1 MONITOR Ultrasensitive test, version 1.5, with a lower limit of detection of 50 copies/ml, currently appears to be the most widely used assay for the quantitation of plasma HIV-1 RNA load [34].

The use of highly active antiretroviral therapy in clinical practice has resulted in a rapid decline of plasma HIV-1 RNA levels that require the ability of NAT to detect minimal plasma HIV-1 RNA concentrations in order for clinicians to evaluate the therapeutic response and make therapeutic decisions [35–37]. Lower HIV-1 RNA plasma levels may be associated with a significantly longer survival, which supports the use of ultrasensitive HIV-1 RNA load assays with a lower limit of detection of 50 copies/ml [38, 39]. Failure to achieve the target level of less than 50 copies/ml after 16 to 24 weeks of treatment should prompt the consideration of drug resistance, inadequate drug absorption, or poor compliance. In general, a significant increase in plasma HIV-1 RNA concentration may indicate the emergence of drug-resistant viral mutants or a lack of adherence to the antiretroviral drug regimen. It must, however, be taken into consideration that inter- and intra-assay variations of molecular assays are relatively great, even if maximum automated assays are employed [9, 10]. For this reason, an increase is only significant if it exceeds half a log compared to the previously obtained value. Furthermore, different assays or even different generations of assays may yield different results [40, 41]. Monitoring of patients should thus always be performed with the identical test. Finally, although the determination of plasma HIV-1 RNA concentration is important, it is not the only parameter to consider in evaluating an antiretroviral drug regimen and making decisions on treatment changes. Further parameters to be taken into account include the CD4+ cell count, progress of clinical disease, unacceptable toxicity or intolerable side effects of drugs, and adherence to the treatment regimen.

Problems to be resolved in the future

The comparison of HIV-1 RNA data generated with different assays remains a critical issue. For patients undergoing anti-HIV therapy, correct quantitation is highly relevant and in blood screening, the lower limit of detection must be defined exactly. The availability of calibrated working reagents would assist in complying with such regulations. In 1999, the First International Standard for HIV-1 RNA (NIBSC code 97/656) for use with NAT was established by the WHO Expert Committee on Biological Standardization and assigned a unitage of 100.000 IU/mL [42]. Have the problems been solved since? In 2004, results of the major commercially available assays are still given in copies/ml and the HIV-1 IU standard does not seem to be accepted. Even very recently introduced and IVD labeled assays such as the COBAS TaqMan assay for HIV-1 shows this lack of consensus. The reason might be that the international standard has several limitations. It just contains a single HIV-1 subtype, is not widely available, and is even contaminated with HBV [43]. Establishing better defined international standards appears to be an important goal in the future.

Currently, there is no commercial assay available for molecular detection of HIV-2. Recently published articles employ in-house assays based on real-time PCR, but none of them introduced an internal control [44–46]. With regard to HIV-1 group O, the situation appears to be similar: Poorly standardized in-house assays lacking internal controls have been described [47–49]. Recently, however, satisfactory results have been obtained with a limited number of samples when using the Abbott LCx quantitative assay [50, 51]. Nevertheless, much improvement is required to obtain an accurate and reliable molecular detection of both HIV-1 group O and HIV-2.

References

1. Clewley JP. The polymerase chain reaction, a review of the practical limitations for human immunodeficiency virus diagnosis. J Virol Methods 1989;25:179–87.
2. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989;339:237–8.

3. Marschner IC, Collier AC, Coombs RW, D'Aquila RT, DeGruttola V, Fischl MA, et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J Infect Dis 1998;177:40–7.
4. Kramvis A, Bukofzer S, Kew MC. Comparison of hepatitis B virus DNA extractions from serum by the QIAamp blood kit, GeneReleaser, and the phenol-chloroform method. J Clin Microbiol 1996;34:2731–3.
5. Shafer RW, Levee DJ, Winters MA, Richmond KL, Huang D, Merigan TC. Comparison of QIAamp HCV kit spin columns, silica beads, and phenol-chloroform for recovering human immunodeficiency virus type 1 RNA from plasma. J Clin Microbiol 1997;35:520–2.
6. Kessler HH, Pierer K, Santner BI, Vellimedu SK, Stelzl E, Marth E, et al. Evaluation of molecular parameters for routine assessment of viremia in patients with chronic hepatitis C who are undergoing antiviral therapy. J Hum Virol 1998;1:314–9.
7. Hoelzl G, Stoecher M, Leb V, Stekel H, Berg J. Entirely automated quantification of human immunodeficiency virus type 1 (HIV-1) RNA in plasma by using the ultrasensitive COBAS AMPLICOR HIV-1 monitor test and RNA purification on the MagNA pure LC instrument. J Clin Microbiol 2003;41:1248–51.
8. Lee BG, Fiebelkorn KR, Caliendo AM, Nolte FS. Development and verification of an automated sample processing protocol for quantitation of human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2003; 41:2062–7.
9. Muller Z, Stelzl E, Bozic M, Haas J, Marth E, Kessler HH. Evaluation of automated sample preparation and quantitative PCR LCx assay for determination of human immunodeficiency virus type 1 RNA. J Clin Microbiol 2004; 42:1439–43.
10. Stelzl E, Baumert R, Haas J, Marth E, Kessler HH. Evaluation of automated sample preparation for quantitative detection of human immunodeficiency virus type 1 RNA. J Lab Med 2004;28:354–60.
11. Murphy DG, Cote L, Fauvel M, Rene P, Vincelette J. 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 human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2000;38:4034–41.
12. Berger A, Rabenau HF, Stief A, Troonen H, Doerr HW. Evaluation of the new LCx HIV RNA quantitative assay: comparison with the Cobas Amplicor HIV Monitor assay. Med Microbiol Immunol 2001;190:129–34.
13. Swanson P, Soriano V, Devare SG, Hackett J. Comparative performance of three viral load assays on human immunodeficiency virus type 1 (HIV-1) isolates representing group M (subgroups A to G) and group O: LCxHIV RNA Quantitative, AMPLICOR HIV-1 MONITOR version 1.5, and Quantiplex HIV-1 RNA version 3.0. J Clin Microbiol 2001;39:862–70.
14. Katsoulidou A, Papachristou E, Petrodaskalaki M, Sypsa V, Anastassopoulou CG, Gargalianos P, et al. Comparison of three current viral load assays for the quantitation of human immunodeficiency virus type 1 RNA in plasma. J Virol Methods 2004;121:93–9.
15. Jagodzinski LL, Wiggins DL, McManis JL, Emery S, Overbaugh J, Robb M, et al. Use of calibrated VL standards for group M subtypes of human immunodeficiency virus type 1 to assess the performance of viral RNA quantitation tests. J Clin Microbiol 2000;38:1247–9.
16. Triques K, Coste J, Perret JL, Segarra C, Mpoudi E, Reynes J, et al. Efficiencies of four versions of the AMPLICOR HIV-1 MONITOR test for quantification of different subtypes of human immunodeficiency virus type 1. J Clin Microbiol 1999;37:110–6.
17. Michael NL, Herman SA, Kwok S, Dreyer K, Wang J, Christopherson C, et al. Development of calibrated viral load standards for group M subtypes of human immunodeficiency virus type 1 and performance of an improved AMPLICOR HIV-1 MONITOR test with isolates of diverse subtypes. J Clin Microbiol 1999;37:2557–63.
18. Dunne AL, Crowe SM. Comparison of branched DNA and reverse transcriptase polymerase chain reaction for quantifying six different HIV-1 subtypes in plasma. AIDS 1997;11:126–7.
19. Nkengasong JN, Kalou M, Maurice C, Bile C, Borget MY, Koblavi S, et al. Comparison of NucliSens and Amplicor Monitor assays for quantification of human immunodeficiency virus type 1 RNA in plasma of persons with HIV-1 subtype A infection in Abidijan, Cote d'Ivoire. J Clin Microbiol 1998;36:2495–8.
20. Damond F, Apetrei C, Descamps D, Brun-Vezinet F, Simon F. HIV-1 subtypes and plasma RNA quantification. AIDS 1999;13:286–8.
21. Jenny-Avital ER, Beatrice ST. Erroneously low or undetectable plasma immunodeficiency virus type 1 (HIV-1) ribonucleic acid load, determined by polymerase chain reaction, in West African and American patients with non-B subtype HIV-1 infection. Clin Infect Dis 2001;32:1227–30.
22. Amendola A, Bordi L, Angletti C, Visco-Comandini U, Abbate I, Cappiells G, et al. Under-evaluation of HIV-1 plasma VL by a commercially available assay in a cluster of patients infected with HIV-1 A/G circulating recombinant form (CRF02). J Acquir Immune Defic Syndr 2002; 31:488–94.
23. Amendola A, Bordi L, Angeletti C, Girardi E, Ippolito G, Capobianchi MR. Comparison of LCx with other current viral load assays for detecting and quantifying human immunodeficiency virus type 1 RNA in patients infected with the circulating recombinant form A7G (CRF02). J Clin Microbiol 2004;42:811–5.
24. Weber B, Fall EMB, Berger A, Doerr HW. Reduction of diagnostic window by new fourth-generation human immunodeficiency virus screening assays. J Clin Microbiol 1998;36:2235–9.
25. Weber B, Berger A, Rabenau A, Doerr HW. Evaluation of a new combined antigen and antibody human immunodeficiency virus screening assay, VIDAS HIV DUO Ultra. J Clin Microbiol 2002;40:1420–6.
26. Weber B, Gürtler L, Thorstensson R, Michl U, Mühlbacher A, Bürgisser P, et al. Multicenter evaluation of a new automated fourth-generation human immunodeficiency virus screening assay with a sensitive antigen detection module and high specificity. J Clin Microbiol 2002;40:1938–46.
27. Roth WK, Weber M, Buhr S, Drosten C, Weichert W, Sireis W, et al. Yield of HCV and IIV-1 NAT after screening of 3.6 million blood donations in central Europe. Transfusion 2002;42:862–8.
28. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975–9.
29. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for

diagnosis and staging of primary HIV infection. AIDS 2003;17:1871–9.
30. Stramer SL, Glynn SA, Kleinman SH, Strong M, Caglioti S, Wright DJ, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. New Engl J Med 2004;351:760–8.
31. Delwart EL, Kalmin ND, Jones TS, Ladd DJ, Foley B, Tobler LH, et al. First report of human immunodeficiency virus transmission via an RNA-screened blood donation. Vox Sang 2004;86:171–7.
32. Mellors JW, Rinaldo CR, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167–70.
33. Saag MS, Holodniy M, Kuritzkes DR, O'Brien WA, Coombs R, Poscher ME, et al. HIV viral load markers in clinical practice. Nat Med 1996;2:625–9.
34. Niesters B on behalf of QCMD and its Working Party on Blood Borne Viruses. Final report. QCMD 2004 Human Immunodeficiency Virus Proficiency Program.
35. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 1997;387:188–91.
36. Raboud JM, Montaner JS, Conway B, Rae S, Reiss P, Vella S, et al. Suppression of plasma viral load below 20 copies/ml is required to achieve a long-term response to therapy. AIDS 1998;12:1619–24.
37. Powderly WG, Saag MS, Chapman S, Yu G, Quart B, Clendeninn NJ. Predictors of optimal virological response to potent antiretroviral therapy. AIDS 1999;13:1873–80.
38. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946–54.
39. Izopet J, Salama G, Pasquier C, Sandres K, Marchou B, Massip P, et al. Decay of HIV-1 DNA in patients receiving suppressive antiretroviral therapy. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:478–83.
40. Lin HJ, Pedneault L, Hollinger FB. Intra-assay performance characteristics of five assays for quantification of human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 1998;36:835–9.
41. Holmes H, Davis C, Heath A, Hewlett I, Lelie N. An international collaborative study to establish the 1st international standard for HIV-1 RNA for use in nucleic acid-based techniques. J Virol Methods 2001;92:141–50.
42. Caliendo AM, Ingersoll J, Green AM, Nolte FS, Easley KA. Comparison of the sensitivities and viral load values of the AMPLICOR HIV-1 MONITOR version 1.0 and 1.5 tests. J Clin Microbiol 2004;42:5392–3.
43. Shyamala V, Cottrell J, Arcangel P, Madriaga D, Phelps B, Chien D. Detection and quantitation of HBV DNA in the WHO International Standard for HIV-1 RNA (NIBSC code: 97/656). J Virol Methods 2004;118:69–72.
44. Schutten M, van den Hoogen B, van der Ende ME, Gruters RA, Osterhaus A, Niesters H. Development of a real-time quantitative RT-PCR for the detection of HIV-2 RNA in plasma. J Virol Methods 2000;88:81–7.
45. Damond F, Descamps D, Farfara I, Telles JN, Puyeo S, Campa P, et al. Quantification of proviral load of human immunodeficiency virus type 2 subtypes A and B using real-time PCR. J Clin Microbiol 2001;39:4264–8.
46. Ruelle J, Mukadi BK, Schutten M, Goubau P. Quantitative real-time PCR on LightCycler for the detection of human immunodeficiency virus type 2 (HIV-2). J Virolog Methods 2004;117:67–74.
47. De Baar MP, van Dooren MW, de Rooij E, Bakker M, van Gemen B, Goudsmit J, de Ronde A. Single rapid real-time monitored isothermal RNA amplification assay for quantification of human immunodeficiency virus type 1 isolates from groups M, N, and O. J Clin Microbiol 2001; 39:1378–84.
48. Ernest I, Alexandre I, Zammatteo N, Herman M, Houbion A, de Leener F, et al. Quantitative assay for group M (subtype A-H) and group O HIV-1 RNA detection in plasma. J Virol Methods 2001;93:1–14.
49. Gueudin M, Plantier JC, Damond F, Roques P, Mauclere P, Simon F. Plasma viral RNA assay in HIV-1 group O infection by real-time PCR. J Virol Methods 2003;113:43–9.
50. Swanson P, Harris BJ, Holzmayer V, Devare SG, Schochetman G, Hackett J. Quantification of HIV-1 group M (subtypes A-G) and group O by the LCx HIV RNA quantitative assay. J Virol Methods 2000;89:97–108.
51. De Mendoza C, Alcami J, Sainz M, Folgueira D, Soriano V. Evaluation of the Abbott LCx quantitative assay for measurement of human immunodeficiency virus RNA in plasma. J Clin Microbiol 2002;40:1518–21.