JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 4234–4236 Vol. 43, No. 8
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.8.4234–4236.2005

Improved Sensitivity of Human Immunodeficiency Virus Type 2 Subtype B Plasma Viral Load Assay

Florence Damond,[1,2]* Gilles Collin,[1,2] Diane Descamps,[1,2] Sophie Matheron,[1] Sophie Pueyo,[3] Audrey Taieb,[3] Pauline Campa,[4] Antoine Benard,[3] Geneviève Chêne,[3] and Francoise Brun-Vezinet[1,2]

Hôpital Bichat Claude Bernard, Paris, France[1]; INSERM U552, Paris, France[2]; INSERM U330, Bordeaux, France[3]; and Hôpital Saint Antoine, Paris, France[4]

Received 27 January 2005/Accepted 23 April 2005

We developed a new assay for human immunodeficiency virus type 2 plasma RNA quantification based on a previous format. The new version performed significantly better than the original regarding the detection of subtype B, allowing the detection of 14 out of 36 plasma RNAs in the subtype B-infected patients not detected with the original version.

Human immunodeficiency virus type 2 (HIV-2) differs from HIV-1 by its lower pathogenicity and lower plasma viral load (3, 12, 13). Two longitudinal studies of patients living in West Africa reported a link between HIV-2 viral load and the CD4 cell count decline and death (2, 11). In the French Agence Nationale de Recherche sur le SIDA HIV-2 cohort study, we confirmed the very slow progression of HIV-2 disease and showed that, as in HIV-1 infection, plasma RNA quantification was predictive of the risk of clinical progression (14).

Another major characteristic of HIV-2 is its high degree of genetic diversity. At least eight HIV-2 subtypes have been described previously (4, 8, 10, 15), and the two major subtypes, A and B, have been identified in France (6). In the absence of an approved commercial assay for HIV-2 RNA quantification, we developed an assay based on a real-time PCR in 2001 (7). The frequency of plasma RNA positivity did not depend on the subtype, but the mean plasma viral load was slightly higher in subtype A infection than in subtype B infection (7). This apparent difference in sensitivity was confirmed by longitudinal studies. Here we developed an improved version of this assay and compared the results with those obtained with the previous format.

We tested EDTA plasma samples stored at −80°C collected from patients included in the French Agence Nationale de Recherche sur le SIDA national cohort, who originate from various African countries and are infected by HIV-2 subtype A ($n = 20$), subtype B ($n = 36$), or subtype H ($n = 1$), as determined by *pol* or env sequencing (6). The new assay differs from the original in two respects: first, RNA is extracted with the Magna Pure automated method (Magna Pure Large Volume; Roche Diagnostic, Meylan, France), and second, a second reverse primer is added (L140, 5′-TCCAACAGGCTCTC TGCTAATCC-3′). Thus, the 20-μl reaction mixes consisted of 7.5 μl of Light Cycler RNA master hybridization mix, 3.25 mM $Mn(OAc)_2$, 0.5 μl of primers F3 and R1 and probe (20 μM), 0.5 μl of primer L140 at 10 μM, and 6 μl of RNA extract. The PCR conditions were as follows: (i) reverse transcription, 61°C for 20 min; (ii) denaturation, 95°C for 2 min; (iii) 45 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 20 s, and elongation at 65°C for 50 s; and (iv) cooling to 40°C for 30 s.

The specificity of the assay was determined by testing plasma samples from 25 HIV-seronegative blood donors and 25 HIV-1-infected patients. The efficiency and sensitivity of real-time reverse transcription-PCR for HIV-2 RNA quantification were determined by testing the HIV-2 RNA standard. No subtype B reference isolates being available, a stock of HIV-2 subtype A strain NIHZ, counted by electron microscopy, was used as a standard (Advanced Biotechnology Incorporated, Maryland). The NIHZ stock solution, corresponding to a subtype A strain, was diluted in HIV-negative plasma to obtain 500,000, 50,000, 5,000, 500, 250, and 100 copies/ml. It was then extracted and treated as described above. To assess the reproducibility of the assay, the standard was diluted in HIV-negative plasma and tested in eight replicates to determine within-run variability and in 10 separate runs to determine between-run variability.

Using automated extraction, the sensitivity of the assay, based on repeated testing (10 replicates each) of the standard concentrations, was 100% at 100 copies/ml. For a theoretical virus concentration of 100 copies/ml, we obtained a mean value of 124 ± 78 copies/ml with a within-run coefficient of variation of 54%. The quantification cutoff for the assay was thus set at 100 copies/ml. None of the HIV-negative or HIV-1-positive samples gave a positive result.

The Light Cycler system offers good reproducibility for HIV-2 RNA quantification. For a theoretical virus concentration of 5.69 $\log_{10}$ copies/ml, we obtained a mean value of 5.65 $\log_{10}$ copies/ml, with a within-run coefficient of variation of 1%. A mean copy number of 5.94 $\log_{10}$ copies/ml was obtained in repeated assays, with a between-run coefficient of variation of 0.44%. At the lowest concentration of 2.39 $\log_{10}$ copies/ml, we found mean values of 2.73 $\log_{10}$ copies/ml and 2.36 $\log_{10}$ copies/ml in within- and between-run assays, with coefficients of variation of 12% and 1.67%, respectively.

Among the 57 HIV-2 plasma samples tested, 28 samples, in which plasma RNA was below 100 copies with the old version,

* Corresponding author. Mailing address: Laboratoire de Virologie, Hôpital Bichat Claude Bernard, 46 rue Henri Huchard, 75018 Paris, France. Phone: 33 1 40 25 61 51. Fax: 33 1 40 25 67 69. E-mail: florence.damond@bch.ap-hop-paris.fr.

TABLE 1. Comparative results of the former assay and the optimized version for HIV-2 viral load for different subtypes

HIV-2 subtype	HIV-2 RNA viral load (copies/ml)		HIV-2 subtype	HIV-2 RNA viral load (copies/ml)	
	Former version	Optimized version		Former version	Optimized version
A	113	<100		<100	5,065
	168	453		<100	17,880
	302	130		<100	20,070
	568	360		<100	21,590
	720	890		<100	27,700
	2,535	1,900		<100	29,930
	3,600	4,935		<100	<100
	3,980	3,055		<100	<100
	4,462	5,559		<100	<100
	5,072	4,056		<100	<100
	5,154	10,170		<100	<100
	8,742	9,957		<100	<100
	<100	179		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
	<100	<100		<100	<100
				<100	<100
B	990	871		<100	<100
	<100	475		<100	<100
	<100	256		<100	<100
	<100	720		<100	<100
	<100	1,300		<100	<100
	<100	1,608		<100	<100
	<100	1,676			
	<100	3,203	H	3,670	8,030
	<100	3,747			

were also undetectable with the new assay (subtype A, $n = 7$; subtype B, $n = 21$). The results are summarized in Table 1. The viral load values given by the former and new versions of our assay for subtypes A and H were not different (medians were 3,790 [original version] and 3,555 [new version] copies/ml and 3,670 [original version] versus 8,030 [new version] copies/ml, $P = 0.89$, for subtypes A and H, respectively). The new version of the test performed significantly better than the original version with regard to the detection of HIV-2 subtype B. Indeed, among the 36 patients infected by B subtype, 14 specimens which gave negative results in the original version were positive with the new version (medians were <100 and 3,475 copies/ml).

This study shows that the automation of RNA extraction by the Magna Pure system leads to increased sensitivity. Moreover, the addition of a new primer does not change the quantification of subtype A or H viral load but clearly improves the quantification of subtype B. Several groups have previously reported the failure of viral load monitoring in patients infected by divergent HIV-1 subtypes (1, 5, 9), and we report here a similar impact of HIV-2 genetic diversity on viral load assay results. Based on these results, it is likely that further refinements of HIV-2 viral load assays will be necessary to encompass the diversity of HIV-2 subtypes.

This study was financially supported by the Agence Nationale de Recherche sur le SIDA.

REFERENCES

1. **Alaeus, A., K. Lidman, A. Sonnerborg, and J. Albert.** 1997. Subtype-specific problems with quantification of plasma HIV-1 RNA. AIDS **11:**859–865.
2. **Ariyoshi, K., S. Jaffar, A. S. Alabi, N. Berry, M. S. van der Loeff, S. Sabally, P. T. N′Gom, T. Corrah, R. Tedder, and H. Whittle.** 2000. Plasma RNA viral load predicts the rate of CD4 T cell decline and death in HIV-2-infected patients in West Africa. AIDS **14:**339–344.
3. **Berry, N., K. Ariyoshi, S. Jaffar, S. Sabally, T. Corrah, R. Tedder, and H. Whittle.** 1998. Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. J. Hum. Virol. **1:**457–468.
4. **Chen, Z., A. Luckay, D. L. Sodora, P. Telfer, P. Reed, A. Gettie, J. M. Kanu, R. F. Sadek, J. Yee, D. D. Ho, L. Zhang, and P. A. Marx.** 1997. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. J. Virol. **71:**3953–3960.
5. **Damond, F., C. Apetrei, D. Descamps, F. Brun-Vezinet, and F. Simon.** 1999. HIV-1 subtypes and plasma RNA quantification. AIDS **13:**286–288.
6. **Damond, F., C. Apetrei, D. L. Robertson, S. Souquiere, A. Lepretre, S. Matheron, J. C. Plantier, F. Brun-Vezinet, and F. Simon.** 2001. Variability of human immunodeficiency virus type 2 (hiv-2) infecting patients living in France. Virology **280:**19–30.
7. **Damond, F., M. Gueudin, S. Pueyo, I. Farfara, D. L. Robertson, D. Descamps, G. Chene, S. Matheron, P. Campa, F. Brun-Vezinet, and F. Simon.** 2002. Plasma RNA viral load in human immunodeficiency virus type 2 subtype A and subtype B infections. J. Clin. Microbiol. **40:**3654–3659.
8. **Damond, F., M. Worobey, P. Campa, I. Farfara, G. Collin, S. Matheron, F. Brun-Vezinet, D. L. Robertson, and F. Simon.** 2004. Identification of a highly divergent HIV-2 and proposal for a change in HIV-2 classification. AIDS Res. Hum. Retrovir. **20:**666–672.
9. **Debyser, Z., E. Van Wijngaerden, K. Van Laethem, K. Beuselinck, M. Reynders, E. De Clercq, J. Desmyter, and A. M. Vandamme.** 1998. Failure to quantify viral load with two of the three commercial methods in a pregnant woman harboring an HIV type 1 subtype G strain. AIDS Res. Hum. Retrovir. **14:**453–459.
10. **Gao, F., L. Yue, D. L. Robertson, S. C. Hill, H. Hui, R. J. Biggar, A. E. Neequaye, T. M. Whelan, D. D. Ho, G. M. Shaw, et al.** 1994. Genetic diversity

of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology. J. Virol. **68:**7433–7447.

11. **Gottlieb, G. S., P. S. Sow, S. E. Hawes, I. Ndoye, M. Redman, A. M. Coll-Seck, M. A. Faye-Niang, A. Diop, J. M. Kuypers, C. W. Critchlow, R. Respess, J. I. Mullins, and N. B. Kiviat.** 2002. Equal plasma viral loads predict a similar rate of CD4+ T cell decline in human immunodeficiency virus (HIV) type 1- and HIV-2-infected individuals from Senegal, West Africa. J. Infect. Dis. **185:**905–914.
12. **Kanki, P. J., K. U. Travers, S. MBoup, C. C. Hsieh, R. G. Marlink, N. A. Gueye, T. Siby, I. Thior, M. Hernandez-Avila, J. L. Sankale, et al.** 1994. Slower heterosexual spread of HIV-2 than HIV-1. Lancet **343:**943–946.
13. **Marlink, R., P. Kanki, I. Thior, K. Travers, G. Eisen, T. Siby, I. Traore, C. C. Hsieh, M. C. Dia, E. H. Gueye, et al.** 1994. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science **265:**1587–1590.
14. **Matheron, S., S. Pueyo, F. Damond, F. Simon, A. Lepretre, P. Campa, R. Salamon, G. Chene, and F. Brun-Vezinet.** 2003. Factors associated with clinical progression in HIV-2 infected patients: the French ANRS cohort. AIDS **17:**2593–2601.
15. **Yamaguchi, J., S. G. Devare, and C. A. Brennan.** 2000. Identification of a new HIV-2 subtype based on phylogenetic analysis of full-length genomic sequence. AIDS Res. Hum. Retrovir. **16:**925–930.