JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2002, p. 3883–3884 Vol. 40, No. 10
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.10.3883–3884.2002

Quantification of Human Immunodeficiency Virus Type 1 Proviral DNA by the TaqMan Real-Time PCR Assay

A variety of real-time PCR assays have been applied for quantification of human immunodeficiency virus type 1 (HIV-1) (4–7). Recently, Désiré et al. have reported a TaqMan PCR for quantification of HIV-1 provirus in peripheral blood mononuclear cells (PBMCs) (3). This method targets the polymerase (*pol*) gene of HIV-1 subtype B. Based on their data, Désiré et al. have concluded that HIV-1 proviral DNA load in PBMCs does not correlate with HIV-1 RNA level in plasma and $CD4^+$ lymphocyte counts. This conclusion is contradictory to those previously drawn by us (2) and recently drawn by others (8). In order to clarify if the use of different DNA quantification methods may have contributed to this controversy, we decided to compare the TaqMan PCR by Désiré et al. with a TaqMan PCR recently developed in our laboratory. Our TaqMan PCR targeted the long terminal repeat region (LTR) of the HIV-1 major group. The sequences of the sense and antisense primers were 5′-GCCTCAATAAAGCTTG CCTTGA-3′ and 5′-GGGCGCCACTGCTAGAGA-3′. The probe sequence was 5′-CCAGAGTCACACAACAGACGGG CACA-3′. The 5′ and 3′ ends of the probe were labeled with FAM and TAMRA dyes. The sensitivity of the assay was estimated to be five copies of HIV-1 plasmid DNA (Applied Biosystems). We randomly selected 25 PBMC samples, which had been earlier confirmed to be HIV-1 DNA positive by a nested PCR (1). The HIV-1 DNA was extracted from the PBMCs with the Qiagen blood kit. Five microliters of each DNA extract (equivalent to 2×10^5 PBMCs) was tested with both the *pol*- and LTR-based TaqMan PCR assays on the same plate for <45 PCR cycles. The threshold cycle numbers (Ct) obtained by the two TaqMan PCR assays are listed in Table 1. Compared with the LTR TaqMan PCR, markedly higher Ct values (>2 cycles) were obtained by the *pol* TaqMan PCR (Table 1). Furthermore, HIV-1 DNA in seven HIV-1-positive PBMC samples was not detected by the *pol* TaqMan assay. Among those seven HIV-1 false-negative samples, three were PBMC culture positive for HIV-1. Our results suggest that the *pol* TaqMan PCR exhibits an unsatisfactory low sensitivity on the selected sample panel. The fact that some of the tested samples belonged to a subtype other than B may partly contribute to the high false negativity of the HIV-1 *pol* TaqMan PCR assay. However, the lower sensitivity was also seen when HIV-1 subtype B SF2 strain was tested with the *pol* TaqMan PCR (Table 1). Therefore, we further examined the *pol* TaqMan PCR design by using PrimerExpress software (version 2.0). The melting temperature of the reverse primer P2 was significantly lower than that of the forward primer P1 (50.4 versus 60.5°C). At the reported annealing temperature (60°C), an asymmetric amplification could have been taking place during PCR due to the poor hybridization of the P2 primer with the template, resulting in decreased amplification efficiency. Considering that HIV-1 load turns out to be extremely low after efficient antiretroviral therapy (ART), we believe that a highly sensitive real-time PCR is demanded for the purpose of ART monitoring.

TABLE 1. Comparison of the numbers of Ct obtained by two TaqMan PCRs for HIV-1 DNA detection in PBMCs

Sample no.	No. of Ct with[a]:		PBMC culture Result
	Assay A	Assay B	
1	30.35	32.35	Positive
2	31.03	33.85	Negative
3	33.68	35.70	Negative
4	32.92	36.34	ND[b]
5	27.40	29.98	ND
6	31.37	39.39	Positive
7	33.21	35.36	Positive
8	34.64	38.34	Positive
9	31.06	42.14	Positive
10	27.01	37.30	Negative
11	39.52	39.85	Negative
12	34.70	38.82	ND
13	37.34	>45	Negative
14	36.87	>45	Positive
15	36.11	39.68	ND
16	33.35	42.50	Positive
17	29.18	39.54	Positive
18	31.49	44.15	Positive
19	32.14	>45	ND
20	33.00	43.45	ND
21	30.00	>45	Positive
22	30.92	>45	Negative
23	34.73	>45	Positive
24	35.46	40.28	Negative
25	33.48	>45	ND
SF2[1c]	18.82	21.77	Positive

[a] Assay A is the TaqMan PCR developed in our laboratory, and assay B is the TaqMan PCR described by Désiré et al. (3).
[b] ND, not determined.
[c] SF2 is an HIV-1 subtype B laboratory strain.

We thank the Swedish Physicians against AIDS Foundation for support.

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