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Evaluation of Viroseq™-HIV Version 2 for HIV Drug Resistance

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We compared a new anti-HIV drug resistance detection kit, Viroseq™-HIV version 2 (AB method, Applied Biosystems, Tokyo), with the standard method developed by the National Institute of Infectious Diseases, Japan (NIID method) for its applicability to subtype E HIV-1.

The tested materials were 24 patients' sera which were found positive for subtype E HIV-1. The subtype was determined by sequencing of C2V3 envelope region. The sera were stock frozen at −80°C, and the freeze-thaw was limited to a maximum of three times.

Detection of mutations using the AB method was performed according to the manufacturer's instructions. In short, RNA was extracted using the guanidine-thiocyanate method from 0.5 ml centrifugation-cleared sera. The 1.7 kb protease-reverse transcriptase region was reverse-transcribed, PCR-amplified in the presence of uracil DNA glycosylase and dUTP, and sequenced by using primers A, B, C, D, F, G, and H (Figure) and a Big-Dye terminator (Applied Biosystems). The nucleotide sequences were analyzed using Sequence Analysis version 3.4, and the drug resistance mutations by HIV genotyping System Software version 2.2. The NIID method has been described elsewhere (1). The differences between the two methods are summarized in Table 1.

First, the sensitivity of the two methods was compared. A patient's serum with HIV-1 titer of 2×10^6 copies/ml (measured by Amplicore HIV MONITOR version 1.5 [Roche Diagnostics, Tokyo]) was diluted serially with HIV-negative serum. The AB method detected HIV-1 genome from all 19 samples with titers higher than $10^{3.4}$ copies/ml. The NIID method detected 18 of the 19 samples. Both methods detected the HIV-1 genome from 2 samples among 5 samples with titers lower than $10^{3.4}$. Therefore, the sensitivities were considered comparable (Table 2).

In the AB method, as internal regions were chosen as primers for sequencing, there was a possibility of mismatch between the primer sequence and some viral template sequences.

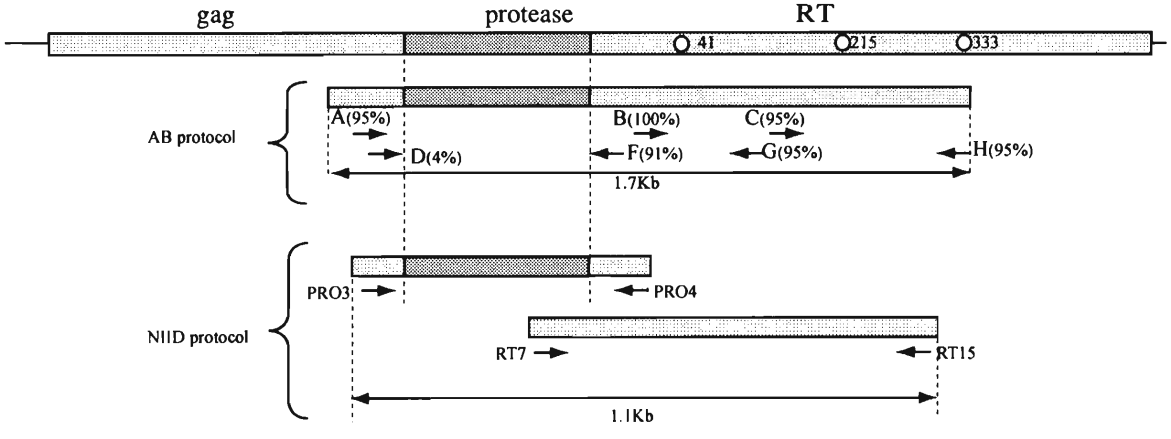

Figure. Primers for sequencing and their efficiency. Percentage in parenthesis indicates percent of successful sequencing.

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Table 1. Comparison of AB method and NIID method

	NIID method	AB method
sample	plasma 50 μl	plasma 500 μl
method	RT+nested-PCR	RT-PCR
RT primer	specific	specific
number of amplicon and each size	2 protease: 450 bp RT: 800 bp	1 pol: 1.7 Kbp
sequence primers	same as the inner PCR primer	specific for sequencing
estimated price per sample	¥8,000	¥20,000

Table 2. Amplification of patient plasma

	HIV-1 RNA LOG10(copies/ml)	Method	
		AB	NIID
JE-1.2	<1.7	–	pro#
JE-2	2.0	–	–
JE-3	3.0	+	pro#
JE-4	3.1	–	+
JE-5.2	3.1	+	+
JE-6.2	3.4	+	+
JE-7	3.4	+	+
JE-6.1	3.4	+	–
JE-8	3.7	+	+
JE-9	3.7	+	+
JE-10	3.9	+	+
JE-11	3.9	+	+
JE-12	4.0	+	+
JE-13	4.3	+	+
JE-14	4.4	+	+
JE-15	4.5	+	+
JE-16	4.6	+	+
JE-17	4.7	+	+
JE-18	5.0	+	+
JE-1.1	5.4	+	+
JE-5.1	5.4	+	+
JE-19	5.6	+	+
JE-20	5.9	+	+
JE-21	6.1	+	+
	% amplification	21/24(87.5%)	22/24(91.7%)

#only protease region was successfully amplified.

Actually, primer D was effective only in 4% of subtype E. However, the sequence of the corresponding region could be obtained by sequencing the complementary strand using a different primer. The efficiency of other primers was quite high, i.e., 100% for primer B, 95% for primers A, C, G, and H, and 91% for primer F.

The drug resistance mutation data obtained using the two methods were compared in 19 cases. The results are summarized in Table 3. As for protease inhibitor resistance, D30N, M46V, G48V, I50V, and I84V were concordant in 100% of the cases, L90M in 94.3%, and V82ATFS in 84.2%. As for nucleotide reverse transcriptase inhibitor resistance, M41L, E44D, K65R, L74V, V118I, Q151M, M184IVT, and L215FY were concordant in 100%, and T69D in 94.7%. As for non-nucleotide reverse transcriptase inhibitor resistance, K103N, V106A, V108I, V118I, Y188CLH, and G190A were all concordant.

Our data showed that the AB method and the NIID method were comparable in regard to sensitivity and the detection of drug resistance mutations. The cost for one sample is currently 8,000 yen (about US$ 80) for the NIID method but for the AB method it is 20,000 yen, more than twofold more expensive than the NIID method. The advantage of the AB method is probably its commercial availability as a kit.

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Table 3. Comparison of drug resistance genotyping results by AB method and NIID method

Sample No.	Method	PI resistance								RTI resistance					% match
		10	54	63	71	77	82	88	90	41	69	75	210	219	
		L	I	L	A	V	V	N	L	M	T	V	L	K	
JE-5.2	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-6.2	AB	–	–	P	V	–	–	–	M	–	–	–	–	–	95.56
	NIID	–	–	P/I	I/V	–	–	–	M	–	–	–	–	–	
JE-7	AB	I	–	–	–	–	–	–	–	L	–	M/I	W	N	97.78
	NIID	I	–	–	–	–	–	–	–	L	–	M	W	N	
JE-8	AB	F	–	–	–	I	–	S	–	–	–	–	–	Q	96.00
	NIID	F	–	–	–	–	–	S	–	–	N	–	–	Q	
JE-9	AB	V	V	–	–	–	F	–	M	L	N/D/A	M	W	Q	86.67
	NIID	V	D/V	–	–	–	F/I	–	M	–	N/D/A	M/I/L	–	–	
JE-10	AB	–	–	–	–	–	I	–	–	L	N	M	–	Q	100.00
	NIID	–	–	–	–	–	I	–	–	L	N	M	–	Q	
JE-11	AB	F	D	–	–	–	–	–	M	L	–	M	W	N	98.00
	NIID	F	V	–	–	–	–	–	M	L	–	M	W	N	
JE-12	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-13	AB	F/I	–	–	–	–	I	–	I/M	–	–	–	–	–	91.11
	NIID	F	–	–	–	–	–	S	–	–	–	–	–	–	
JE-14	AB	Y	–	–	–	–	–	–	–	–	–	I	–	–	96.00
	NIID	–	–	–	–	–	–	–	–	–	–	I	M	–	
JE-15	AB	F	–	–	–	–	–	S	–	L	–	–	W	–	98.00
	NIID	F	–	–	T	–	–	S	–	L	–	–	W	–	
JE-16	AB	–	–	P	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	P	–	–	–	–	–	–	–	–	–	–	
JE-17	AB	–	–	P	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	P	–	–	–	–	–	–	–	–	–	–	
JE-18	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-1.1	AB	–	–	–	–	–	D	–	–	–	–	–	–	–	98.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-5.1	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-19	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-20	AB	–	–	–	–	–	–	–	–	–	–	–	–	–	100.00
	NIID	–	–	–	–	–	–	–	–	–	–	–	–	–	
JE-21	AB	I	–	–	–	–	I	–	–	–	–	–	–	–	100.00
	NIID	I	–	–	–	–	I	–	–	–	–	–	–	–	
														average	97.74

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