



Performance of the TRUGENE *HIV-1* gp41 Analyte-Specific Reagents (ASR)



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ABSTRACT

Background: Commercially available HIV-1 genotyping kits sequence the PR- and RT-coding regions of *pol*. The TRUGENE *HIV-1* gp41 ASR, with the OpenGene DNA Sequencing System, was designed to detect drug resistance mutations in the gp41-coding segment of *env* selected by the fusion inhibitor enfuvirtide (ENF). Assay performance was evaluated using a panel of plasma samples obtained from ENF-naïve and -experienced HIV-1-infected subjects. **Methods:** Fifty-one plasma samples were obtained from 26 subjects (20 prior to ENF and 31 during or after ENF exposure). HIV-1 RNA was extracted using a Qiagen viral RNA extraction minikit. Samples were then subjected to an RT-PCR and the HR-1 region of *env* sequenced using the TRUGENE HIV-1 gp41 ASR or a home-brew assay using an ABI 3700 PRISM automated sequencer. Each pair of sequences obtained was then manually aligned with each other and with a subtype B reference sequence (NL4-3), and differences between the two test sequences were scored to determine the percent agreement. Mutations in the region including codons 36-45 were scored as possible ENF-resistance mutations.

Results: Sequence data were obtained by both methods for 43 samples. No sequence could be obtained for 7 samples (14%) by the home brew method and for 1 sample (2%) by the TRUGENE assay. The mean number of ENF resistance mutations per sample was 1.0 (range, 0-2). Mean percent agreement across a shared 231-nucleotide (nt) region encompassing HR-1 was 99.4% (range, 97.4-100%). Mean percent agreement at the amino acid level at codons 36-45 was 97.7% (range, 80-100%). Sequence data from multiple independent clones (n=7-14) were available for 31 samples with a valid TRUGENE result. Overall, there was excellent agreement between the population-based sequences obtained by the TRUGENE and home-brew methods and sequences obtained from clones.

Conclusion: The TRUGENE *HIV-1* gp41 ASRs and the OpenGene DNA Sequencing System generated highly accurate sequence data when tested with plasma samples as compared to home-brew and clonal sequence analysis.

METHODS

Blood plasma was collected from 26 HIV-1-seropositive subjects undergoing enfuvirtide (ENF) treatment. 20 specimens were obtained prior to ENF administration and 31 post-ENF exposure, with HIV-1 RNA levels in plasma ranging from 3.12 – 6.30 log₁₀ copies per ml. Using the QIAGEN QIAamp Viral RNA Mini kit, viral RNA was isolated from 140µl of the HIV-1 infected patient plasma. The extracted viral RNA specimens were then stored at -70°C, at which point a TRUGENE *HIV-1* gp41 ASR and home-brew assay were performed.

Following the manufacturer's recommendations in the TRUGENE *HIV-1* gp41 ASR assay (ASR), 10µl of extracted viral RNA was subjected to an RT-PCR, containing primers specific to amplifying a gp41 and gp120-containing region of the *env* gene. A CLIP reaction was then conducted to sequence and detect mutations in the HR1 region. Sequence data was acquired and analyzed using the OpenGene DNA Sequencing System. Alternatively, the home-brew assay involved the use of 7.5µl of the extracted RNA specimen in an RT-PCR with primers to amplify *env*. The resultant cDNA amplicons were sequenced using an ABI 3700 PRISM automated sequencer. The sequence fragments obtained for each specimen were then manually aligned with the sequence generated from the ASR assay, and a subtype B (NL4-3) reference sequence. Analysis involved the percent agreement between the 2 methods at the nucleotide and amino acid levels across a 231-bp region of HR1. A detailed assessment of codons 36-45 was performed, which comprises the region in which typical mutations conferring resistance to ENF are found. For clonal analysis, an *env* segment spanning HR-1 and HR-2 was amplified in multiple independent PCRs and at least 7 independent clones were sequenced from each sample. The assessment included a comparison of TRUGENE data to data obtained by clonal analysis for 31 of the 50 specimens successfully sequenced by the ASR assay.

RESULTS

TABLE 1. Accuracy of the TRUGENE HIV-1 gp41 Analyte-Specific Reagents

Comparison	% Agreement	% Mismatches due to mixtures
Nucleotides 43-273	99.4	94.9 (n=56)
Amino acid level		
Codons 15-91	99.1	100 (n=31)
Resistance codons (aa 36-45)	97.7	100 (n=10)

TABLE 2. All incompletely identified codons at resistance positions ^a

Specimen ID	Patient ID	Codon detected by ASR	Codon detected by home-brew
09	3502	N42NT	N42
11	3083	G36D/G	G36D
11	3083	V38M/V	V38
12	3097	G36G/V	G36V
16	3506	Q40H/Q	Q40
31	3505	Q40H	Q40Q/H
32	3507	L44M/L	L44
45	3511	N42NT	N42T
47	3520	N43K/N	N43
49	3520	L44M/L	L44M

^a Excludes N42S, which is a polymorphism that does not confer ENF resistance.

TABLE 3. Comparison of resistance site mutations by ASR to clonal analysis ^a

Specimen ID	Patient ID	Detected by ASR	Detected by Clonal Analysis ^b
01	3019	V38E,N42S	V38E
05	3151	G36D/G,N43N/D	N43D 7/7
06	3174	V38A/V,Q40Q/H,N42T,L45M/L	V38A,N42T 8/8
08	3501	N42N/S	N42S 1/9
09	3502	V38A,N42N/T	V38A 7/7
13	3501	WT	WT
14	3504	N43D	N43D
16	3506	Q40H/Q,N43N/D	N42S 4; Q40H 2; L45Q 1
18	3508	N42S	Q40H 2; N43D 8
21	3501	Q40H,N42S	Q40H,N42S 4/8; N42S 2/8; WT 2
22	3505	G36E/G	G36E 5; WT 4
23	3507	WT	WT
27	3519	WT	WT
31	3505	Q40H,L45M/L	Q40H,L45M 4; G36E 2; G36R 1
32	3507	V38A/V,N42N/T,L44M/L,L45Q/L	V38A,L44M 4; V38A,N42T 2
33	3514	N42S	WT
34	3516	WT	WT
35	3518	N43D	N43D 11/11
36	3520	WT	WT
37	3521	N42S	N42S 8/8
38	3518	N43D	N43D 14/14
39	3521	V38A,N42S	V38A 6; I37M,V38A 1; V38A,Q39R 1
40	3518	N43D	N43D 8/8
42	3171	V38A/V,Q39H,N42T,L44M	Q39H,N42T,L44M 4; V38A,Q39H,L44M 2; Q39H,N42T 2
43	3507	V38A/V	V38A 10; V38A,N42D 1
44	3508	N42S,N43D	WT 5; N43D 3
46	3514	V38A,N42S	V38A 9/9
47	3520	G36D/G,N43K/N	G36D 5; Q40H 1
49	3520	V38A,L44M/L	V38A,L44M
50	18879221	G36V,N42N/S	G36V 6/8
51	3516	WT	WT

^a RED = mutations common to both methods, BLACK = mutations detected by ASR as a mixture

^b numbers denote the number of clones in which the mutation(s) was found (n=7-14)

CONCLUSIONS

The TRUGENE *HIV-1* gp41 ASR is a robust and accurate assay for detecting mutations throughout the gp41 HR1 region of *env*, particularly those that confer resistance to ENF.

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