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Automation of the ViroSeq HIV-1 Genotyping Assay Using the Abbott m2000sp and NanoDrop 2000

Anita McClernon[1], Andrew Freeman[1], Danijela Lucic[2], Gavin Cloherty[2] and Daniel McClernon[1]

[1] bioMONTR, Research Triangle Park, NC, [2]Abbott Molecular, Inc., Des Plaines, IL

Introduction

- HIV-1 resistance testing is standard of care for HIV-1 patient management.
- The FDA approved ViroSeq HIV-1 genotyping assay utilizes manual ETOH sample extraction and gel electrophoresis for amplicon quantitation.
- Herein we describe the performance of the ViroSeq HIV-1 assay when used with the Abbott m2000sp for automated nucleic acid extraction and NanoDrop 2000 spectrophotometer for amplicon quantitation.

Method

- Comparative genotypic analysis was performed on HIV-1 infectious plasma samples (n = 16) of varying viral loads ranging from ~3,800 c/mL to ~148,000 c/mL.
- The first aliquot was analyzed using the FDA approved ViroSeq HIV-1 Genotyping System v2.0 (Abbott Molecular, Des Plaines, IL). All nucleic acid extracts were stored at -20ºC pending RT-PCR. Repeat analysis was required for 2 aliquots; these nucleic acid extracts were stored at -80ºC pending RT-PCR.
- Nucleic acid from the second aliquot was extracted using a modified Total Nucleic Acid (TNA) protocol on the automated m2000sp instrument followed by analysis using the ViroSeq assay. All nucleic acid extracts were stored at -20ºC pending RT-PCR. Repeat analysis was required for 2 aliquots; these nucleic acid extracts were stored at -80ºC pending RT-PCR.
- Prior to cycle sequencing, PCR products were normalized based on concentrations obtained using a NanoDrop 2000 spectrophotometer.
- A subset of manual and m2000sp-derived amplicons were analyzed for correct size and density on an Agilent 2100 bioanalyzer.
- Sequence analysis was performed using an ABI 3100 Genetic Analyzer.
- HIV-1 sequence concordance was analyzed via bioMONTR's proprietary bioConT sequence analysis tool.

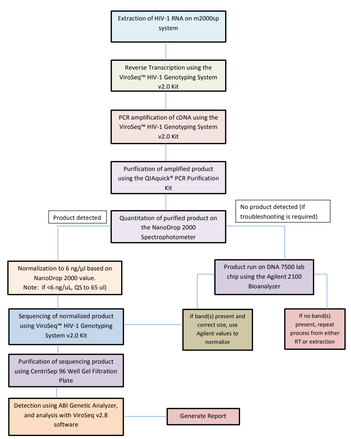

Figure 1 ViroSeq HIV-1 assay workflow diagram.

Results

Sample Information					Viral Load		NanoDrop Values	Drug Resistance Mutations			Concordance based on Drug Resistance Mutations	Concordance at the Nucleotide Level
Sample	Level	Replicate	Assay	Dilution Factor	c/mL	LOG c/mL	purified PCR product (ng/ul)	NRTI	NNRTI	PI		
1	1	1	ETOH ViroSeq	1	148,140	5.17	19	No Mutations Identified			100%	99.77%
		3	m2000sp ViroSeq				2.7	No Mutations Identified				
	2	1	ETOH ViroSeq	1:2	74,080	4.87	32.1	No Mutations Identified			100%	99.92%
		3	m2000sp ViroSeq				8	No Mutations Identified				
	3	1	ETOH ViroSeq	1:4	37,040	4.57	24.3	No Mutations Identified			100%	99.92%
		3	m2000sp ViroSeq				3.8	No Mutations Identified				
	4	1	ETOH ViroSeq	1:8	18,520	4.27	3.5	No Mutations Identified			100%	99.77%
		3	m2000sp ViroSeq				3.2	No Mutations Identified				
2	1	1	ETOH ViroSeq	1	134,424	5.13	18.3	No Mutations Identified			100%	99.69%
		3	m2000sp ViroSeq				11.8	No Mutations Identified				
	2	1	ETOH ViroSeq	1:2	67,212	4.83	48.8	No Mutations Identified			100%	99.77%
		3	m2000sp ViroSeq				12	No Mutations Identified				
	3	1	ETOH ViroSeq	1:4	33,606	4.53	18.5	No Mutations Identified			100%	99.69%
		3	m2000sp ViroSeq				8.9	No Mutations Identified				
	4	1	ETOH ViroSeq	1:8	16,803	4.23	16.7	No Mutations Identified			100%	99.85%
		3 repeat	m2000sp ViroSeq				9	No Mutations Identified				
3	1	1	ETOH ViroSeq	1	15,176	4.18	11.6	M41L, E44D, D67N, **L74I**, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M	mixtures	99.00%
		3	m2000sp ViroSeq				6.1	M41L, E44D, D67N, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M		
	2	1 repeat	ETOH ViroSeq	1:2	7,588	3.88	13.3	M41L, E44D, D67N, **L74I**, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M	mixtures	99.08%
		3	m2000sp ViroSeq				5.3	M41L, E44D, D67N, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M		
	3	1	ETOH ViroSeq	1:4	3,794	3.58	15.7	M41L, E44D, D67N, **L74I**, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M	mixture	98.92%
		3	m2000sp ViroSeq				1.6	M41L, E44D, D67N, **L74V**, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M		
4	2	1	ETOH ViroSeq	1:2	28,400	4.45	4.1	M41L, T215Y			100%	98.54%
		3	m2000sp ViroSeq				5.2	M41L, T215Y				
	4	1 repeat	ETOH ViroSeq	1:8	7,100	3.85	9.4	M41L, T215Y			100%	98.23%
		3	m2000sp ViroSeq				2.5	M41L, T215Y				
5	1	1	ETOH ViroSeq	1	24,336	4.39	7.1	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M	100%	99.54%
		3	m2000sp ViroSeq				6	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M		
	2	1	ETOH ViroSeq	1:2	12,168	4.09	2.4	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M	100%	98.00%
		3	m2000sp ViroSeq				2.3	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M		
	3	1	ETOH ViroSeq	1:4	6,084	3.78	4	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M	100%	99.16%
		3 repeat	m2000sp ViroSeq				6.7	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M		

Table 1 ViroSeq HIV-1 assay with manual extraction and m2000sp extraction.

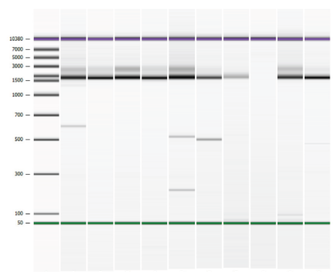

Figure 2 Manual extraction: Lane 1 (18 ng/ul), 3 (49 ng/ul), 5 (18 ng/ul), 7 (4 ng/ul) and 9 (17 ng/ul); m2000sp extraction: Lane 2 (12 ng/ul), 4 (12 ng/ul), 6 (9 ng/ul), 8 (1.7 ng/ul) and 10 (9 ng/ul). Lane 1 & 2 represent sample 2-1; Lane 3 & 4 represent sample 2-2; Lane 5 & 6 represent sample 2-3; Lane 7 & 8 represent sample 5-3; Lane 9 & 10 represent sample 2-4. NOTE: Cycle Sequencing was not performed on the PCR product represented in Lane 8; this sample was re-extracted and successfully sequenced.

Results Continued

A

B

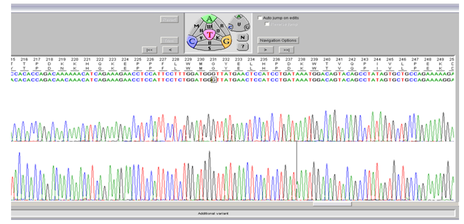

Figure 2 ViroSeq HIV-1 assay with (a) m2000sp extraction and (b) manual extraction.

- Nucleic Acid extracts stored for more than 2 days at -20ºC yielded poor amplification and/or low sequence signal. Repeat testing was required (n = 4 samples) and extracts were stored at -80ºC pending subsequent analysis.
- HIV-1 drug resistance mutations demonstrated 100% concordance for 13/16 pairs between manual and m2000sp-automated extractions.
- PCR products analyzed on the Agilent 2100 bioanalyzer were the correct size and density at ~1.8kb.
- A mixture of L74I/V (A/T/G) was identified in 3/16 samples using the manual method while the corresponding m2000sp samples reported a mixture of L74V (T/G).

Conclusions

- The Abbott m2000sp extraction platform and NanoDrop spectrophotometer can be utilized in combination with ViroSeq HIV-1 Drug Resistance assay.
- For optimal performance, nucleic acid extracts should be stored at -80ºC.
- The use of the Abbott m2000sp and NanoDrop increases productivity and turnaround time for HIV-1 drug resistance assay.

Send correspondence to:
Daniel McClernon
dmcclernon@biomontr.com

bioMONTR