MULTICENTER CLINICAL TRIAL STUDY OF THE SENTOSA SQ HIV GENOTYPING ASSAY, A NEXT-GENERATION SEQUENCING TEST

MAYO CLINIC

Cleveland Clinic

VELA DIAGNOSTICS

J. T. Morken[1], C. T. Irish[1], D. Kohn[2], L. Doyle[2], J. Fischer[3], K. Shaut[3], B. J. Digmann[1], J. J. Germer[1], E. Wee[4], I. Ng[4], C. Lee[4], D. Henton[3], G. W. Procop[2], B. Yen-Lieberman[2], J. D. C. Yao[1]

[1]Mayo Clinic, Rochester, MN; [2]Cleveland Clinic, Cleveland, OH; [3]Vela Diagnostics USA, Inc., Fairfield, NJ; [4]Vela Diagnostics Singapore Pte. Ltd., Singapore.

Abstract no. Tuesday-01
35th Annual Clinical Virology Symposium
Savannah, GA; May 5 - 8, 2019

ABSTRACT *(Revised)*

Background: Antiviral drug resistance testing is a standard of care in the management of individuals with HIV-1 infection. Currently, the Sanger sequencing-based ViroSeq™ HIV-1 Genotyping System, v2.0 (ViroSeq; Abbott Molecular Systems, Inc.) is the only HIV-1 antiretroviral drug resistance detection assay approved by FDA for clinical use in the U.S. We conducted a multicenter FDA registration trial to evaluate the performance characteristics of a new, next-generation sequencing assay, the *Sentosa*® SQ HIV Genotyping Assay (Sentosa HIV; Vela Operations Singapore Pte. Ltd.).

Methods: For limit of detection (LoD), an HIV-1 group M subtype B reference strain was tested in 20 replicates each at 500, 1,000, and 2,000 copies/mL with Sentosa HIV, while group M subtypes A, C, D, F, G, H, J, and K (3 strains each) were tested at 1,000 copies/mL to confirm the LoD across these various subtypes. HIV-1 group M subtype B strain preparations containing known drug resistance mutations (DRM) at frequencies of 5%, 10%, 15%, 20%, and 40% were also tested in 60 replicates each at 1,000 copies/mL to assess LoD for DRM. Additionally, preparations containing DRM at frequencies of 5% and 10% were tested in 60 replicates each at 5,000, 15,000, and 20,000 copies/mL. Analytical reproducibility was evaluated by testing replicates of HIV-1 subtypes A, B, C, and D both individually (each at 3,000 copies/mL) and in subtype mixtures A+D, B+D, C+D (each at 45,000 copies/mL) in 30 assay runs performed across 3 laboratory sites, 3 reagent kit lots, 3 instrument systems, and 6 operators. Clinical reproducibility was also assessed at each of 3 testing sites using replicate panels containing 20 clinical plasma specimens (each at 4,000 copies/mL) with known HIV-1 DRM. Clinical sensitivity and specificity of Sentosa HIV were assessed by testing 107 retrospectively collected clinical plasma specimens with HIV-1 RNA levels ranging from 4,330 to 10,000,000 copies/mL and known DRM (previously detected with ViroSeq and/or a laboratory-developed Vela Integrase assay).

Results: LoD (≥95% rate) was 1,000 copies/mL for HIV-1 group M and confirmed among the other subtypes tested. At 1,000 copies/mL, DRM present at frequencies ≥20% were detected in ≥90% of replicates, while DRM present at ≤15% were detected in <90% of replicates. Additionally, DRM at frequencies of 5% were only detectable at HIV-1 RNA levels ≥15,000 copies/mL. Analytical reproducibility was 100% among the 30 assay reproducibility runs, with overall DRM detection rates of 99.6%, 98.1%, and 97.9% across the 3 testing sites (96.0% κ-coefficient) and an inter-assay %CV of 4.44% at a 5% DRM frequency. Clinical reproducibility yielded valid HIV-1 sequences in 179 of 180 replicates, with 98.2% to 100% of expected DRM detected across the 3 testing sites (98.7% κ-coefficient) when DRM present at <10% frequency were excluded. All 107 clinical plasma specimens yielded valid sequences with Sentosa HIV with sensitivity and specificity of 96.2% and 99.9%, 95.6% and 99.9%, and 96.1% and 99.9% for PR / RT, INT, and overall DRM detection, respectively, when excluding DRM detected at <20% frequency (for direct comparison to Sanger sequencing-based methods).

Conclusion: As a semi-automated, sample-to-answer, next-generation sequencing assay, Sentosa HIV provides sensitive and reproducible detection of genotypic antiretroviral drug resistance mutations among HIV-1 group M strains found in clinical plasma specimens.

INTRODUCTION

The *Sentosa*® SQ HIV Genotyping Assay (Sentosa HIV) is a new, next-generation sequencing (NGS) assay intended for use in the detection of HIV-1 genotypic drug resistance mutations (DRM) occurring in the protease (PR), reverse transcriptase (RT), and integrase (INT) regions of HIV-1 recovered from the plasma of infected individuals. It is the first commercial, semi-automated, sample-to-result, NGS assay designed for this purpose and is intended for use with the *Sentosa*® SX101, and SQ301 instruments. Sentosa HIV is specifically designed to interrogate 2 different regions of the HIV-1 genome: ~1,500 bp (PR and RT codons 1 to 99 and 1 to 337, respectively) and ~1,000 bp (INT codons 1 to 288).

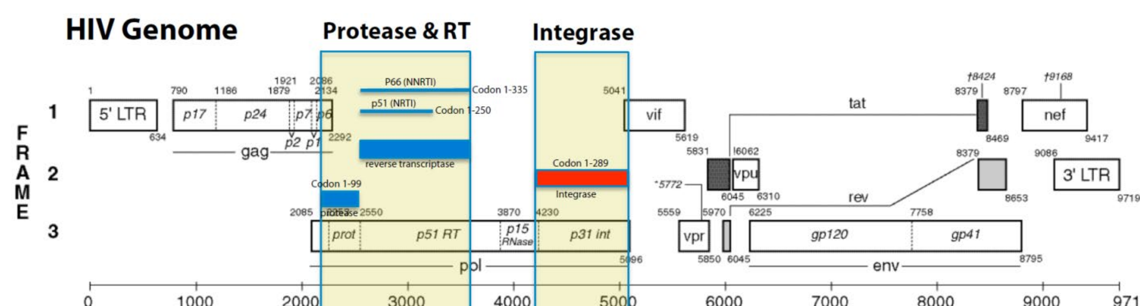

Figure 1: Workflow for Sentosa HIV

Day	Steps		Instruments/Software	Hands-on Time	Instrument Time
Day 1	**1) Extraction**	Lysis, binding, washing, and elution	SX101	30 min	2 hrs, 15 min
Day 1	**2) Library preparation**	RT-PCR preparation			
Day 1		RT-PCR	Veriti® Dx Thermal Cycler	5 min	2 hrs, 20 min
Day 1		Normalization, shearing, purification, and ligation	SX101	10 min	4 hrs
Day 2		Library pooling		-	15 min
Day 2	**3) Template preparation**	Isothermal amplification, ISP enrichment	SX101	15 min	2 hrs, 30 min
Day 2	**4) Sequencing**	Initialization and sequencing	SQ301	1 hr	5 hrs
Day 2	**5) Data analysis**	Signal processing, base calling, alignment, and variant calling	SQ Reporter	NA	4 hrs
				<2 hrs Hands-on Time	

METHODS

Limit of Detection (LoD):

- HIV-1 group M subtype B reference strain, 20 replicates each at 500, 1,000, and 2,000 copies/mL;
- HIV-1 group M subtypes A, C, D, F, G, H, J, and K (3 strains each), 20 replicates each at 1,000 copies/mL;
- HIV-1 group M subtype B preparations with known DRM frequencies of 5%, 10%, 15%, 20%, and 40% (each at 1,000 copies/mL) along with 5% and 10% at 5,000, 15,000, and 20,000 copies/mL; all tested in 60 replicates each.

Analytical / Clinical Reproducibility:

- HIV-1 group M subtypes A, B, C, and D were used to prepare replicates of individual subtypes at 3,000 copies/mL and subtype mixtures A+D, B+D, and C+D (1:19 ratios) at 45,000 copies/mL for testing in 10 assay runs performed by each of 3 laboratory sites using different reagent lots, instrument systems, and 2 different operators;
- Panels containing 20 de-identified clinical plasma specimens with HIV-1 at 4,000 copies/mL and with known DRM were tested in triplicate at each of 3 laboratory sites with 3 different reagent lots.

Clinical Sensitivity / Specificity:

- 107 clinical plasma specimens with HIV-1 RNA levels ranging from 4,330 to 10,000,000 copies/mL and known DRM (previously determined by ViroSeq and a laboratory-developed Vela Integrase assay) were tested at 3 laboratory sites.

Table 1: Comparison of Results among Clinical Specimens (*n* = 107)

PR / RT		ViroSeq (reference method)					
		Exclusion of DRM at <20%			No exclusions		
		DRM	WT	Total	DRM	WT	Total
Sentosa HIV	DRM	689	30	719	705	103	808
	WT	27	28,499	28,526	27	28,499	28,526
	Total	716	28,529	29,245	732	28,602	29,334
	Sensitivity	96.2%	(689 / 716)		96.3%	(705 / 732)	
	Specificity	99.9%	(28,499 / 28,529)		99.6%	(28,499 / 28,602)	

INT		Vela Integrase (reference method)					
		Exclusion of DRM at <20%			No exclusions		
		DRM	WT	Total	DRM	WT	Total
Sentosa HIV	DRM	130	2	132	133	8	141
	WT	6	7,015	7,021	6	7,015	7,021
	Total	136	7,017	7,153	139	7,023	7,162
	Sensitivity	95.6%	(130 / 136)		95.7%	(133 / 139)	
	Specificity	99.9%	(7,015 / 7,017)		99.9%	(7,015 / 7,023)	

PR / RT and INT		ViroSeq + Vela Integrase (reference method)					
		Exclusion of DRM at <20%			No exclusions		
		DRM	WT	Total	DRM	WT	Total
Sentosa HIV	DRM	819	32	851	838	111	949
	WT	33	35,514	35,547	33	35,514	35,547
	Total	852	35,546	36,398	871	35,625	36,496
	Sensitivity	96.1%	(819 / 852)		96.2%	(838 / 871)	
	Specificity	99.9%	(35,514 / 35,546)		99.7%	(35,514 / 35,625)	

PR, protease; RT, reverse transcriptase; INT, integrase; DRM, drug resistance mutation; WT, wild type.

RESULTS

- LoD (95% detection rate) of 1,000 copies/mL was established for HIV-1 group M and confirmed with subtypes A, C, D, F, G, H, J, and K.
- At 1,000 copies/mL, DRM present at frequencies ≥20% were detected in ≥90% of replicates, while DRM present at ≤15% were detected in <90% of replicates.
- DRM detection at a 5% frequency required HIV-1 RNA levels of ≥15,000 copies/mL.
- Analytical reproducibility of 100% was observed among 10 assay runs performed at each of 3 laboratory sites, with overall DRM detection rates of 99.6%, 98.1%, and 97.9% among the 3 sites (96.0% κ-coefficient).
- Overall inter-assay %CV of 4.44% at 5% DRM frequency level.
- 98.2% to 100% of expected DRM were detected among 20 clinical reproducibility specimens tested in triplicate at each of the 3 laboratory sites when DRM at a frequency of <10% were excluded (98.7% κ-coefficient).
- Clinical sensitivity and specificity were 96.2% and 99.9%, 95.6% and 99.9%, and 96.1% and 99.9% for PR / RT, INT, and overall DRM detection, respectively, when DRM at a frequency of <20% were excluded from analysis (for direct comparison to Sanger sequencing-based methods).

CONCLUSION

As a semi-automated, sample-to-answer, next-generation sequencing assay, Sentosa HIV provides sensitive and reproducible detection of genotypic antiretroviral drug resistance mutations among HIV-1 group M strains found in clinical plasma specimens.

©2018 Mayo Foundation for Medical Education and Research