Evaluation of the Vela DX system for determining HIV-1 genotypic resistance and comparison with direct sequencing and NGS platforms

Poster 97

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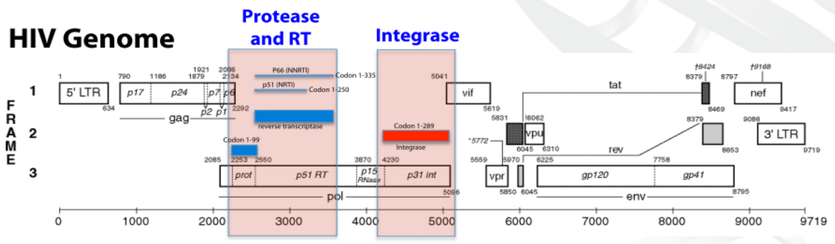
Background: The aim of this study was to evaluate the diagnostic performance of the Vela Sentosa next-generation sequencing system in conjunction with the Sentosa SQ HIV Genotyping Assay for sequencing and genotyping HIV-1 samples.

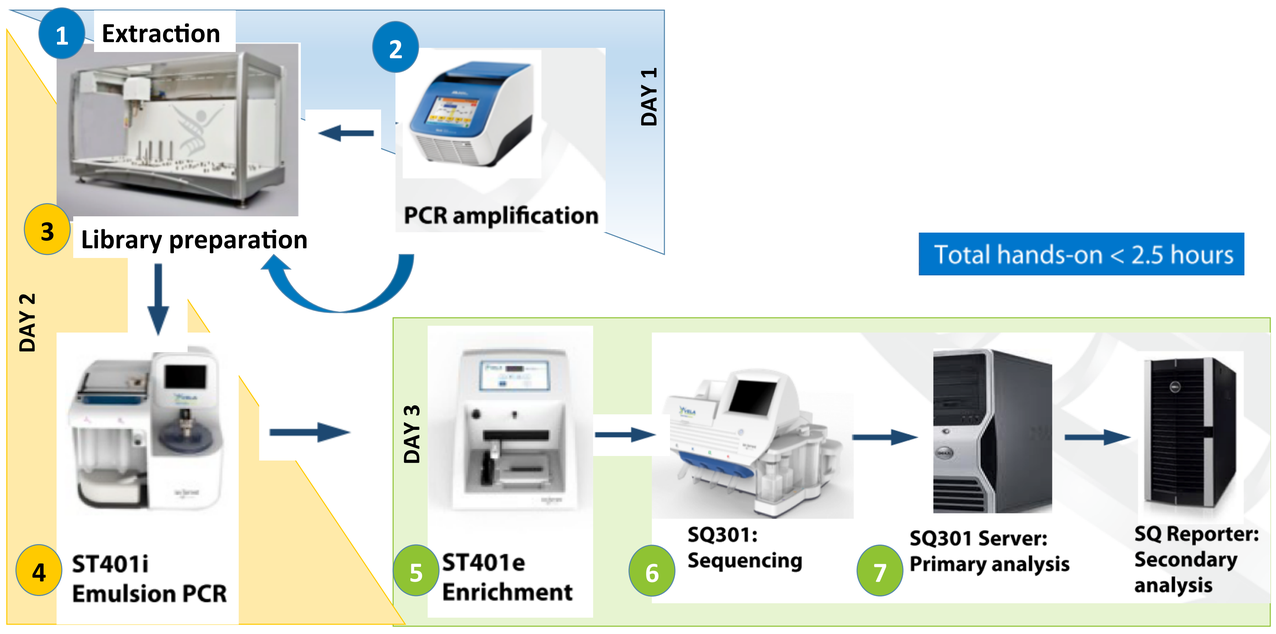
Materials and methods

Plasma samples were extracted and templates prepared on the Sentosa SX instrument. Reverse transcriptase (RT), protease (PR) and integrase genes were sequenced on the Sentosa SQ 301 Sequencer (PGM Ion Torrent). Sequence analyses and lists of mutations were provided by the Vela software. HIV-1 specimens of various subtypes harboring resistance-associated mutations were used to validate all analyses. Methods were compared using 46 clinical samples (mean viral load: 6.15 log copies/mL) that had been characterized by direct sequencing and on two NGS platforms, 454 GS-Junior Roche or MiSeq Illumina.



- Protease+RT: K03455 : 2251 – 3784 (Protease codons 1-99, RT codons 1-411)
- Integrase: K03455 : 4162 – 5071 (Codons 1-280)
- Amplicons length: ~1500 bp (Protease+RT) and ~1000 bp (Integrase)

Vela next generation sequencing workflow



Total hands-on < 2.5 hours

Results

Table 1. Analytical sensitivity for subtype B and CRF02-AG

	HIV-1 subtype B							HIV-1 CRF02-AG				
Viral loads cp/mL	100000	10000	2000	1000	500	200	100	100000	10000	2000	1000	500
PR-RT Mean coverage	5356	5768	2278	6588	6657	2787	12699	3939	6001	6394	4801	3694
Number of triplicate	3	3	3	3	3	3	1	3	3	3	3	3
IN Mean coverage	12219	11197	7787	10530	9341	12812	9794	15160	9784	9461	6264	8100
Number of triplicate	3	3	3	3	3	2	3	3	3	3	3	3
Mutations unexpected			PR 54T 6,38% *		RT 219R 13% *					IN 230N 3,97% *		

* Mutation observed in one of the triplicate (not found on the fastq file)

Two samples of subtype B and CRF02-AG with majority resistance mutations in PR and RT were diluted with negative HIV plasma for evaluating the analytical sensitivity (Table 1). All the triplicates were amplified and the mutations were correctly identified up to 500 cp/mL. The unexpected mutations were not present in the fastq files but generated after data analysis.

Results – Analytical performance

The analytical sensitivity for detecting major resistance mutations in HIV-1 subtype B samples was 200 copies/mL; it was 500 copies/mL for CRF02-AG specimens (Table 1). Minor variants were detected with a sensitivity of 5% at 100,000 copies/mL (Figure 1). The sequences of reference HIV-1 strains (A, B, C, D, F, G, CRF01-AE and CRF02-AG) were concordant with those obtained by direct sequencing (Table 2). Reproducibility was measured on a mixture (10,000 copies/mL) containing 20% mutated variants; the within-run coefficient of variation was 13% and the between-run coefficient was 33%. No sample cross-contamination was observed.

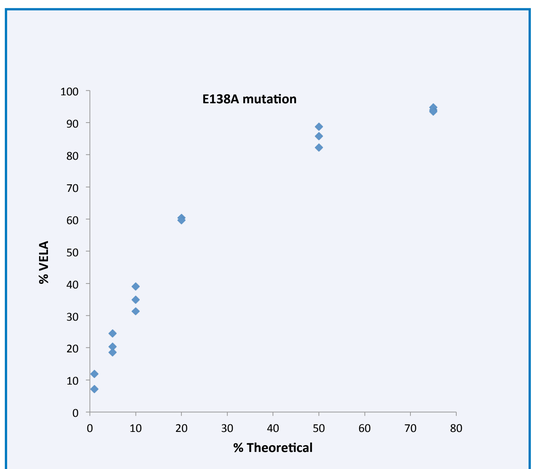


Figure 1. Sensitivity for detecting minor variants. One sample with major resistance mutation (RT E138A) was mixed with JRCSF to obtain proportions of 75%, 50%, 20%, 10%, 5% and 1% of resistance mutation at the concentration of 100 000 copies. Each mixture was tested in triplicate.

E138A mutation (RT) was detected in 3/3 triplicates up to 5% and in 2/3 at 1%. The same observation was done for the mutations M184V (RT) and V77I (PR).

Table 2. Performance of Vela NGS sequencing on HIV-1 reference strains

Subtype (NIH strain)	Vela subtype	Sanger PR mutations	Vela PR mutations (frequency)	Sanger RT mutations	Vela RT mutations (frequency)
B (BaL)	B	V77I	V77I (99%)	-	-
B (JRFL)	B	-	-	K103R V179I	K103R (99%) V179I (99%)
B (ADA)	B	-	-	M41L	M41L (99%)
A	A1	L33F M36I L89M	L33F (99.5%) M36I (99.5%) L89M (99.8%)	V179I	V179I (99.7%) K103E (12.6%)
C	CRF07-BC	D60E L63P I93L	D60E (99.8%) L63P (99.8%) I93L (99.8%)	-	-
D	D	-	-	-	-
F	F1	M36I/V L63L/P L89I/M	M36I (47.3%)/36V (53.9%) L89M (99.9%)	-	-
G	G	K20I M36I V82I L89M	K20I (98.8%) M36I (99.5%) V82I (99.9%) L89M (99.9%)	62wt/V	62V (42.83%)
CRF01-AE	CRF01-AE	G16E K20R M36I L89M	G16E (6.4%) K20R (99.5%) M36I (99.8%) L89M (99.1%)	V179I	V179I (99.7%) K238R (99.9%)
CRF02-AG	CRF02-AG	K20I M36I L89M	K20I (99.3%) M36I (99.6%) V77I (7.7%) L89M (99.8%)	-	-

Table 3. Concordance between Vela NGS system and Sanger direct sequencing for the detection of HIV resistance mutation

	Mutations	Sanger (N mutations)	Vela (N mutations)
Protease	10V	5	3
	16E	6	6
	20I	10	10
	36I	17	17
	62V	6	6
	63P	17	17
	69K	5	5
	71T	2	2
	71V	2	2
	77I	6	6
	89M	13	13
	93L	4	4
Reverse Transcriptase	41L	1	1
	90I	1	1
	103N	1	1
	138A	2	2
	179I	2	2
	179D	1	1
	215D	1	0
	215C	1	1

Results – Methods comparison

The Vela DX system detected 100/103 mutations identified by direct sequencing (concordance 97%)*. Vela DX identified 3/17 mutations, accounting for 1-20% of the quasipecies identified by MiSeq and 50/50 mutations >20% (23 clinical samples) (Table 4). Vela DX also identified 3/27 mutations, accounting for 1-20% of the quasispecies identified by the 454 GS-Junior and 61/64 of the >20% mutations (43 clinical samples) (Table 5). The Vela DX and 454 GS-Junior quantified 66 mutations (Spearman correlation ρ=0.6898; p<0.0001; mean difference: 2.3% by Bland Altman plot). The Vela DX and MiSeq quantified 53 mutations (Spearman correlation ρ=0.4060; p=0.0026; mean difference: 1.1%) (Figure 2).

*The three mutations not listed on the Vela report were present in the fastq files.

Table 4. Concordance Vela/MiSeq in 23 PR-RT samples

Mutation frequency	MiSeq	Vela	Frequency of Vela detection (%)
1-5%	14	3	21
5-20%	3	0	0
>20%	50	50	100

Table 5. Concordance Vela/454 in 43 PR-RT samples

Mutation frequency	454 GS-FLX	Vela	Frequency of Vela detection (%)
1-5%	22	1	5
5-20%	5	2	40
>20%	64	61	98

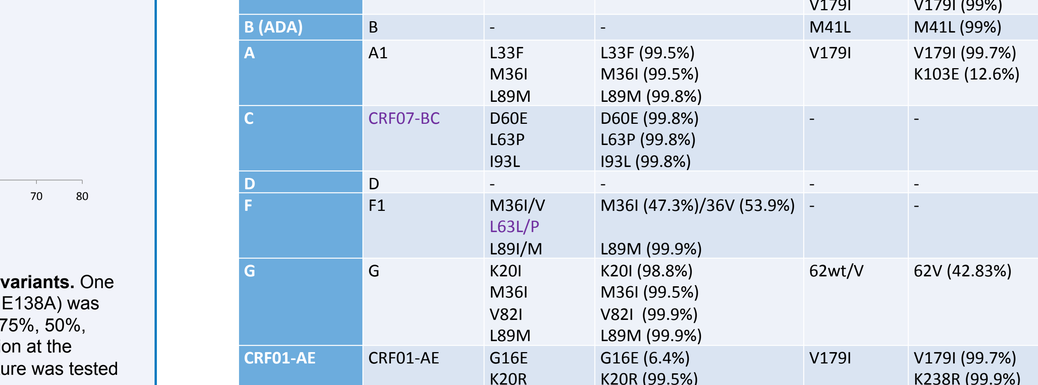


Figure 2. Comparison of two NGS methods for the quantification of the mutated variants.
Bland-Altman diagram comparing Vela NGS System and another NGS platform (454 GS-FLX and MiSeq). Each point represents one of the mutated variant quantified by both platforms. The bias is the mean of the difference between both methods. The 95% limit of agreement contains 95% of the possible differences.

Conclusion

- ❖ **The Vela DX system and HIV Genotyping Assay accurately identified HIV-1 genotype resistance mutations**
- ❖ **Nucleic acid extraction, PCR reagent distribution, library preparation and bio-informatic analysis are all automated**
- ❖ **Vela DX identified the same resistance-associated mutations as those found by direct sequencing**
- ❖ **The three NGS platforms, Vela DX, 454 GS-Junior and MiSeq, all detected variants accounting for more than 20% of the quasispecies**

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