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New HIV Genotyping Assay based on Next Generation Sequencing for HIV Drug Resistance Testing

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Background: Since live saving treatment with antiretroviral HIV drugs became possible in 1987, the emergence of resistance to antiretroviral drugs has become a major life threatening concern. Although HIV treatment has improved dramatically with respect to number, potency and availability of drugs the analysis of drug resistance mutations (DRMs) remains of high clinical importance. Since the first commercially available HIV drug-resistance tests were launched in-house assays underwent significant improvements. The emergence of next generation sequencing (NGS) platforms allowed parallel deep sequencing of clinically relevant regions with high accuracy. Here we present validation data from the first commercially available NGS-based HIV genotyping assay specifically developed for routine diagnostic use in comparison to in-house genotyping analysis (Sanger and NGS).

Methods: We used the *Sentosa*® SQ HIV Genotyping Assay (Vela Diagnostics) covering the HIV protease (PR), reverse transcriptase (RT) and integrase (IN) genes. The system comprised of 1) a robotic liquid handling system for RNA extraction and NGS library preparation; 2) Ion Torrent-based NGS system; 3) kits for RNA extraction, HIV NGS library preparation and sequencing, and 4) data analysis and reporting software. The Vela system allows sequence data export for usage of alternative data interpretation systems. For comparison all samples were analysed with our in-house HIV genotyping system using Sanger and NGS for genotyping. Subtype prediction of HIV Vela system was compared to our in-house system using the COMET HIV-1 subtyping tool. Resistance interpretation of the HIV VELA system as well as the exported sequence data were compared to an in-house Sanger and NGS-based Illumina MiSeq analysis using the HIV-GRADE interpretation system.

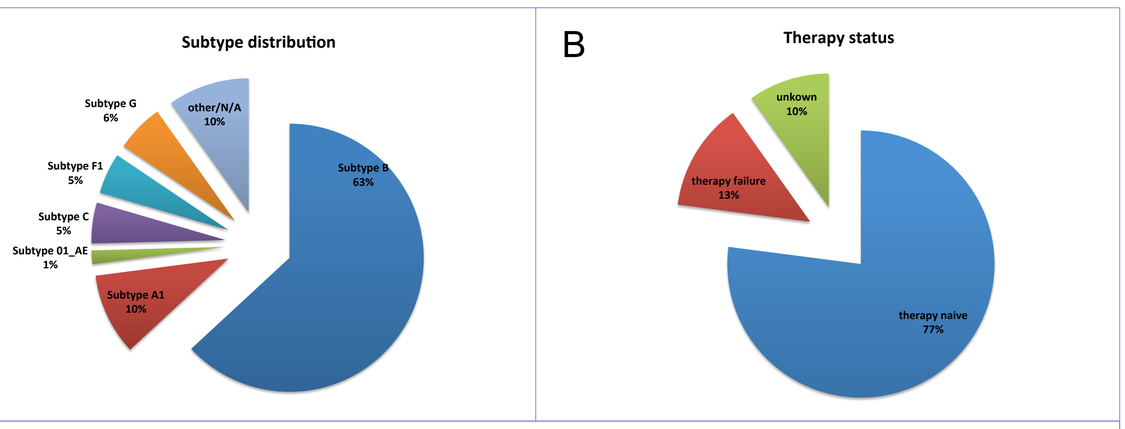

Fig. 2. A Subtype distribution of the PR-RT and IN samples amplified with in-house HIV genotyping system and subtyped with the COMET HIV-1 subtyping tool.
B Patients therapy status of the analysed blood samples.

Steps		Instruments/Software	Hands-on Time	Instruments Time	
1) Extraction	Plasma / Serum extraction	 SX101	30 minutes	2 hours 15 minutes	Day 1
2) Library preparation	RT-PCR preparation				
	RT-PCR	 Thermal Cycler	5 minutes	4 hours 20 minutes	
	Normalization, shearing and ligation	 SX101	15 minutes	3 hours 45 minutes	Day 2
3) Template preparation	Emulsion PCR	 ST401i ST401e	15 minutes	5 hours 30 minutes	
	ISPs enrichment		15 minutes	35 minutes	
4) Sequencing	Machine initialization and sequencing	 SQ301	1 hour	5 hours	Day 3
			Total hands-on < 2.5 hours		
5) Data analysis	Signal processing and variant calling	 SQ Reporter	NA	4 hours	

Fig. 1. Overview of *Sentosa*® SQ HIV Genotyping Assay workflow

NRTI DRMs			PI DRMs		
DRM	Concordance %	Vela/Sanger/NGS	DRM	Concordance %	Vela/Sanger/NGS
M41L	96.33	5 / 2 / 4	D30N	98.17	2 / 2 / 2
D67N	96.33	4 / 4 / 4	M46I	97.25	2 / 1 / 1
K70R	99.08	3 / 3 / 2	M46L	97.25	3 / 2 / 0
M184I	88.07	0 / 0 / 13	I50L	99.08	1 / 0 / 0
M184V	98.17	3 / 3 / 3	I54T	99.08	0 / 0 / 1
L210W	100.00	1 / 1 / 1	I54V	99.08	0 / 0 / 1
T215F	99.08	0 / 0 / 1	V82A	99.08	0 / 0 / 1
T215Y	99.08	1 / 0 / 1	V82F	98.17	0 / 0 / 2
K219E	97.25	2 / 2 / 2	V82L	98.17	2 / 2 / 0
K219Q	98.17	2 / 1 / 0	N88D	98.17	2 / 2 / 2

NNRTI DRMs			INI DRMs		
DRM	Concordance %	Vela/Sanger/NGS	DRM	Concordance %	Vela/Sanger/NGS
K101E	98.17	1 / 1 / 3	Q148R	99.08	0 / 0 / 1
K103N	96.33	3 / 2 / 1			
K103S	98.17	1 / 1 / 3			
E138G	99.08	1 / 1 / 0			
E138K	100.00	1 / 1 / 1			
E138Q	99.08	1 / 1 / 0			
E138A	95.41	5 / 5 / 0			
Y181C	99.08	2 / 2 / 1			
Y181I	98.17	3 / 1 / 1			
Y188C	98.17	0 / 0 / 2			
M230L	97.25	0 / 0 / 3			

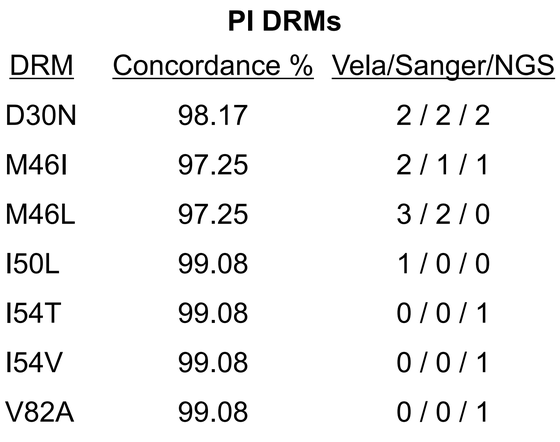

Fig. 3. Concordance of the single detected NRTI, NNRTI, PI and INI DRMs between the three methods Vela system, in-house Sanger sequencing and in-house NGS with concordance (central column) and the absolute number of the detected mutations (right column). The diagram describes proportion of the detected DRMs by each system.

Results: We analysed 122 plasma samples with a viral load of more than 1000 copies/mL of therapy naive and therapy experienced patients with 3 different HIV genotyping methods. Not all samples could be amplified, however 109 samples could be successfully analysed with all three methods.

With regard to subtype prediction between Vela versus COMET HIV-1 subtyping based on Sanger sequencing we saw a concordance of 98.2% within PR-RT and 99.1% within IN.

The number of detected DRMs in the samples was low due to high proportion of therapy naive patients (77%; Fig. 2B) derived from our RESINA study. For therapy experienced patients (13%; Fig. 2B) we could demonstrate that the Vela system detects all mutations which were found by Sanger sequencing. On the contrary Vela reported additional mutations (>3.4% minorities) compared to Sanger, 5 for NRTI, 3 for NNRTI and 2 for PI. A 2.6% minority Q148R (IN) was reported in one sample by Illumina MiSeq NGS and not by Vela or Sanger.

Six PI mutations (1x 46I, 3x 46L, 1x 50L and 2x 82L), four NRTI (1x 41L, 1x 70R and 2x 219Q) and eight NNRTI (2x 103N, 1x 138G, 1x138Q, 5x 138A and 1x 181C) were found with the Vela system and not with in-house NGS. The NRTI mutation M184I was detected only (n=13) with in-house NGS. Notably this mutation was detected in total NA samples (Nucleid Acid extraction from whole blood) which was only performed in our in-house NGS approach. Further proviral analysis and comparison with Vela and in-house NGS, both from buffy coat samples, are needed to verify the relevance of the detection of M184I. However, the mutation is discussed to be APOBEC induced and not clinical relevant.

The high quality of detection of DRMs in TE and TN patients for Vela and in-house NGS is clearly demonstrated here and is in line with previous reports.

Conclusions: The Vela system is on the level of other NGS systems and robust for the daily diagnostic with a high level of concordance (Vela vs. Sanger vs. in-house NGS 97.64%).