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► To cite this version:

Stéphanie Raymond, Florence Nicot, Florence Abravanel, Luce Minier, Romain Carcenac, et al.. Performance evaluation of the Vela Dx Sentosa next-generation sequencing system for HIV-1 DNA genotypic resistance. *Journal of Clinical Virology*, 2020, 122, pp.104229. 10.1016/j.jcv.2019.104229 . hal-03155829

HAL Id: hal-03155829

<https://ut3-toulouseinp.hal.science/hal-03155829v1>

Submitted on 21 Jul 2022

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1 **Performance Evaluation of the Vela Dx Sentosa Next-Generation Sequencing**
2 **System for HIV-1 DNA Genotypic Resistance**

3

4 Running title: NGS for HIV-1 DNA genotyping

5

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23 Abstract word count: 246

24 Text word count: 1300

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27 **Highlights**

- 28 • HIV-1 DNA resistance genotyping may help guiding treatment simplification
- 29 • We compared the Sentosa NGS assay with Sanger sequencing for DNA
- 30 genotyping
- 31 • Automated DNA extraction and NGS accurately predicted HIV DNA drug
- 32 resistance
- 33 • Further investigation should clarify the clinical impact of resistance in DNA

34 **Abstract**

35 **Background:** Patients on antiretroviral therapy could benefit from HIV-1 DNA
36 resistance genotyping for exploring virological failure with low viral load or to guide
37 treatment simplification. Few new generation sequencing data are available.

38 **Objective:** To check that the automated deep sequencing Sentosa platform (Vela
39 DX) detected minority resistant variants well enough for HIV DNA genotyping.

40 **Study design:** We evaluated the Sentosa SQ HIV genotyping assay with automated
41 extraction on 40 DNA longitudinal samples from treatment-experienced patients by
42 comparison with Sanger sequencing. HIV drug resistance was interpreted using the
43 ANRS algorithm (v29) at the threshold of 20% and 3%.

44 **Results:** The Sentosa SQ HIV genotyping assay was 100% successful to amplify
45 and sequence PR and RT and 86% to amplify and sequence IN when the HIV DNA
46 load was >2.5 log copies/million cells. The Sentosa and Sanger sequencing were
47 concordant for predicting PR-RT resistance at the threshold of 20% in 14/18 samples
48 successfully sequenced. A higher level of resistance was predicted by Sentosa in
49 three samples and by Sanger in one sample. The prevalence of resistance was 7%
50 to PI, 59% to NRTI, 31% to NNRTI and 20% to integrase inhibitors using the Sentosa
51 SQ genotyping assay at the threshold of 3%. Seven additional mutations $<20\%$ were
52 detected using the Sentosa assay.

53 **Conclusion:** Automated DNA extraction and sequencing using the Sentosa SQ HIV
54 genotyping assay accurately predicted HIV DNA drug resistance by comparison with
55 Sanger. Prospective studies are needed to evaluate the clinical interest of HIV DNA
56 genotyping.

57 **Keywords:** next-generation sequencing; drug resistance; reverse transcriptase;
58 integrase; DNA genotyping

59 **1. Background**

60 Human immunodeficiency virus (HIV) genotypic resistance testing is recommended
61 before treatment initiation and to optimize antiretroviral treatment after treatment
62 failure [1]. Testing usually involves Sanger sequencing or deep sequencing of
63 plasma samples to detect minority resistant variants (<15-20%) [2–6]. Sequencing of
64 proviral DNA can provide useful information for individuals with a low virus load at the
65 time of virological failure or with undetectable plasma HIV RNA and no previous
66 genotype to guide treatment simplification [7–9]. Drug-resistant mutations identified in
67 DNA can predict virological rebound in patients switching treatments [8]. The lack of
68 automation of Illumina sequencing platforms limits its use for HIV genotyping in
69 clinical practice [10,11]. The semi-automated Sentosa platform (Vela DX) has been
70 developed for routine HIV-1 genotyping on plasma RNA [12–14] but a recent study
71 has shown a high failure rate of the runs [15]. Thus, the performance of an
72 automated extraction and HIV DNA genotyping must be assessed in the context of
73 an increasing routine use of those tests.

74

75 **2. Objective**

76 We aimed to evaluate the performance of the Sentosa next-generation sequencing
77 (NGS) system for genotyping HIV DNA samples by comparison with Sanger
78 sequencing. We also evaluated an external DNA automated extraction before
79 introduction of nucleic acids on the platform.

80

81 **3. Study design**

82 We tested longitudinal samples from 40 patients with HIV-1 infection treated at the
83 Toulouse University Hospital for HIV DNA genotypic resistance. The plasma HIV

84 RNA was below 30 copies/mL (Aptima HIV-1 Quant Dx assay; Hologic, Roissy,
85 France) (35) or below 200 copies/mL (5) at the time of genotyping.

86 HIV-1 DNA was extracted from 250 μ L of buffy coat (PBMC-enriched blood
87 fraction) using the DNA and Viral NA Small Volume Kit on the MagNA Pure 96
88 instrument (Roche Diagnostics, Meylan, France). The 100 μ L eluate was used for
89 parallel amplification and sequencing with Sanger and Sentosa genotyping (Vela
90 Diagnostics, Hamburg, Germany) techniques. Cell-associated HIV-1 DNA was
91 quantified by real-time PCR with the Generic HIV DNA CELL assay kit (Biocentric,
92 Bandol, France) as previously described [16]. The Sentosa SQ HIV Genotyping
93 Assay generated two amplicons, PR-RT (protease-reverse transcriptase) and IN
94 (integrase). The limit of detection of the assay is 5% at 15,000 copies/mL and 20% at
95 1,000 copies/mL; all the mutations identified by the Sentosa SQ reporter (>3%) were
96 considered for the analysis. The PR and RT sequences were obtained by Sanger
97 sequencing using the ANRS protocol ([http://www.hivfrenchresistance.org/ANRS-
98 procedures.pdf](http://www.hivfrenchresistance.org/ANRS-procedures.pdf)). We used the ANRS resistance algorithm (2018, v29, available at
99 <http://www.hivfrenchresistance.org>) to identify resistance-associated mutations
100 (RAMs) after NGS and Sanger sequencing. Categorical variables were tested by the
101 Fisher's exact test.

102 We first used HIV-1 DNA samples with known concentrations to estimate the
103 success rate of genotyping (Table 1).

104

105 **4. Results**

106 The HIV-1 subtype distribution was: 26 B, 6 CRF02-AG, 1 A6, 1 F1, 1 URF (unique
107 recombinant form) and 5 unknowns. The PR-RT amplification and genotyping was
108 72.5% (29/40) successful using the Vela platform and 45% (18/40) successful using

109 Sanger sequencing. We compared the RAMs identified at the threshold of 20% (NGS
110 20%) for 18 samples that have been sequenced by both methods (Table 2). The
111 level of drug resistance predicted by Sanger and NGS 20% was similar in 14/18
112 samples. A higher level of resistance was predicted by NGS 20% in three samples
113 and by Sanger in one sample ($P=0.6$). Five RAMs detected only by NGS accounted
114 for more than 20% of the virus quasispecies (138K 24%, 184I 28%, 179I 65%, 41I
115 90% and 230I 95%). The positions and frequencies of mutations are shown in Figure
116 1. The prevalence of resistance to at least one protease inhibitor (PI) was 7%
117 whatever the genotyping method. The prevalence of resistance to at least one
118 nucleos(t)ide RT inhibitor (NRTI) was 59% and to at least one non-NRTI (NNRTI)
119 was 31% using NGS at the threshold of 3%. Seven additional RAMs accounting for
120 less than 20% of the virus quasispecies (138K 3%, 115F 4%, 215F 4%, 230I 9%, 41L
121 12%, 101E 18% and 184V 19%) were detected using NGS.

122 We assayed integrase resistance genotyping only by NGS; it was 75%
123 successful. The prevalence of resistance to INSTI was 20% (6 samples) according to
124 the ANRS algorithm (Table 3). Three patients had been treated with integrase
125 inhibitors while the other three patients harboured polymorphic mutations (one 157Q
126 and one 74M) [17,18] and a commonly observed G-to-A hypermutation (118R) [19].
127 The patients 046_15 and 042_16 had an undetectable viral load despite resistance to
128 INSTI probably because they received three more active drugs according to the DNA
129 genotype.

130 A modification on the Sentosa program enabled us to use DNA extracts that are
131 added at the step of PCR set-up by the instrument. The automated DNA extraction
132 and Vela NGS took 4 hours to handle 15 samples and the results were obtained in 3

133 days while Sanger sequencing took 7 hours to handle and the results were obtained
134 in 2 days.

135

136 **5. Discussion**

137 We found that a HIV DNA load of at least 1.6 log copies/ 10^6 cells was sufficient for
138 resistance genotyping using the Sentosa NGS platform with success rates of 82%
139 and 94% for the PR-RT and IN regions, respectively. As the median HIV DNA was
140 2.5 log copies/ 10^6 cells (IQR, 2.1-2.9) in a cohort of patients on suppressive
141 antiretroviral therapy [20], HIV DNA genotyping should be successfully performed in
142 patients eligible for a regimen change.

143 The Vela platform was more successful (72.5%) than Sanger sequencing (45%)
144 for detecting resistance in the PR-RT region in DNA samples from our 40 patients.
145 The success rate was close to the one previously published (80%) using the 454 GS-
146 FLX [21]. The Vela platform and Sanger sequencing performed similarly for
147 identifying RAMs at the threshold of 20%. NGS provided identification of RAMs below
148 20% and determination of the proportion of resistant variants in the quasispecies.
149 These mutations were associated with antiretroviral resistance to at least one PI in
150 7% of patients, to at least one NRTI in 59%, and to at least one NNRTI in 31% using
151 the threshold of 3%. The prevalence of resistance was similar according to ANRS
152 and IAS algorithms. Our results were consistent with exposure of these patients to
153 antiretroviral drugs and with a previous study that found 40.5% of resistance to NRTI
154 and 21.6% to NNRTI in HIV DNA using Sanger sequencing [9]. Our prospective
155 study included 40 DNA samples over one year during which a total of 45 runs were
156 performed and 18% failed because of technical problems, as previously described by
157 another group [15]. For the first time, we optimized automated nucleic acid extraction

158 on the MagNA Pure 96 instrument for HIV DNA genotyping on the Sentosa NGS
159 platform. Only one study evaluated this platform without automation of nucleic acid
160 extraction [22]. Automation reduces hands-on time and risk of errors but we showed
161 that it failed to improve the sequencing performance of the three regions PR, RT and
162 IN (success rate of 65% in our study versus 58% in the study by Alidjinou et al.).

163 Prevalence of INSTI resistance was 20% in HIV DNA samples which could
164 influence the choice of drugs for treatment simplification [23–26]. This prevalence
165 was similar using the IAS list (17%) that differed only by the absence of the E157Q
166 mutation (other differences had no impact on resistance). One limitation of our study
167 is that we did not compare NGS and Sanger sequencing of the integrase. Nor did we
168 analyze patient follow-up data to determine the impact of the RAMs on the virological
169 response to combined therapy.

170 We conclude that the Vela NGS platform is suitable for automation of HIV-1
171 DNA deep sequencing, including the nucleic acid extraction, and provides valuable
172 information about drug resistance in patients eligible for treatment simplification when
173 prior genotypic data are not available. Further investigation is needed to clarify the
174 clinical impact of resistance in cellular DNA.

175

176

177 **Funding**

178 This research did not receive any specific grant from funding agencies in the public,
179 commercial, or not-for-profit sectors.

180

181 **Transparency declaration**

182 The authors declare no competing interest.

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- 311

312

313 **FIGURE**314 **Figure 1. Resistance mutations identified using the Vela NGS platform and**315 **Sanger sequencing.** Mutations responsible for resistance to at least one

316 antiretroviral drug according to the ANRS algorithm are represented on the graph.

317 The number of times each mutation occurs is indicated on the ordinate. The first bar

318 stands for the Vela platform and the second bar stand for Sanger sequencing. Three

319 categories of mutation frequency were distinguished with the Vela platform: below

320 5%, between 5 and 20%, and over 20% of the virus quasispecies. The asterisk

321 indicates the mutations that are not listed by the International AIDS Society (IAS;

322 <https://www.iasusa.org/resources/hiv-drug-resistance-mutations/>).

323 **TABLES**324 **Table 1. Impact of HIV-1 DNA load on the global success rate of the Vela NGS for resistance genotyping**

	HIV-1 DNA load		
	<1.5 log cp/10 ⁶ cells (N=3)	1.6-2.5 log cp/10 ⁶ cells (N=10)	>2.5 log cp/10 ⁶ cells (N=7)
PR genotyping	0 (0%)	7 (70%)	7 (100%)
RT genotyping	0 (0%)	7 (70%)	7 (100%)
IN genotyping	1 (33%)	10 (100%)	6 (86%)

325

326

327 **Table 2. Resistance mutations identified by Sanger and NGS methods**

ID region	Subtype	Sanger and NGS RAMs	Sanger RAMs alone	NGS RAMs alone >20%	NGS RAMs 5%-20%	NGS RAMs <5%	Resistance Sanger vs NGS at 20%
Samples with more RAMs using NGS at 20% threshold							
277-PR,RT	B	PR: 10I 36I 54V 62V 63P 71V 82A 90M 93L RT: 62V 67N 70R/S/K/N 184V/M 215F 219Q		PR: 20R 73S RT: 69D 179I		PR: 33F RT: 70G	Sanger = NGS
283-PR,RT	B	PR: 63P 77I RT: 69N 184V 190A		RT: 69A/D		RT: 101E	Sanger = NGS
257-RT	A6			RT: 41I 184I 230I			Sanger < NGS
Samples with more RAMs using Sanger							
708-PR,RT	B	PR: 63P 77I 93L	PR: 20R		PR: 62V	RT: 215F 219Q	Sanger = NGS
432-RT	B		RT: 184V 227Y		RT: 184I 230I		Sanger = NGS
454-PR,RT	B	PR: 36I 62V 63P	PR: 10I				Sanger = NGS
241-PR,RT	B	PR: 63P 77I 93L	PR: 20R 30N 33V 82I RT: 210I				Sanger > NGS
Samples with discordances using Sanger and NGS							
373-PR,RT	B	PR: 36I RT: 106I	PR: 77G				Sanger = NGS
556-PR,RT	B	PR: 36I 62V 63P RT: 41L 210W 215D	PR: 77M	PR: 93L RT: 90I 179I			Sanger < NGS
615-PR,RT	URF	PR: 20I 36I	PR: 15V 73S RT: 230I	PR: 35G 64M	RT: 184V		Sanger = NGS
992-PR	CRF02	PR: 20I 64L	PR: 16R 30N 60N 86M	PR: 36I			Sanger = NGS
693-PR,RT	CRF02	PR: 16E 20I 36I RT: 103R	PR: 77I	RT: 138K 184I			Sanger < NGS
Samples with concordant RAMs at 20% threshold							
941-PR,RT	B	PR: 63P			PR: 36I RT: 230I	RT: 115F 138K 184I	Sanger = NGS
657-RT	F1				RT: 41L 70R 215C		Sanger = NGS
422-PR,RT	B	PR: 63P 77I 93L RT: 215D/E 181C/Y 98S 215D/E			RT: 70R 215C	RT: 215A	Sanger = NGS
497-PR,RT	B	PR: 36I 63P 64V 93L RT: 215L					Sanger = NGS
177-PR	CRF02	PR: 10I 20I 36I 63P					Sanger = NGS
335-PR,RT	B	PR: 36I					Sanger = NGS

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329 ^asample identification and region sequenced by Sanger and NGS (PR for protease, RT for reverse transcriptase)330 ^bRAMs: resistance associated mutations

331 **Table 3. Virological description of six patients harbouring resistance to INSTI**

Patient	HIV-1 subtype	INSTI RAMs ^a	Drug resistance (ANRS) ^b	Ongoing treatment ^c	Previous INSTI exposure
001_14	B	E138A 24% G163R 11%	DTGqd, BIC	TDF+FTC+DRV/r	RAL /EVG
046_15	B	G140S 42% G163R 71% G163K 27%	RAL, EVG, DTGqd, BIC	TDF+FTC+DRV+ RAL	RAL
042_16	B	G140S 66%	RAL, EVG, DTGqd, BIC	ABC+3TC+RPV+ DTG	EVG / DTG
029_16	CRF02	E157Q 96% L74M 99%	RAL, EVG, DTGqd, BIC, CAB	TDF+FTC+DRV/r	None
044_15	Non B	E157Q 100%	RAL, EVG, DTGqd, BIC, CAB	TDF+FTC+ATV/r	None
040_15	CRF02	G118R 5%	RAL, EVG, DTG, BIC	TDF+FTC+EFV	None

332 ^aRAMs: resistance-associated mutations with the frequency in the virus population; ^bRAL=raltegravir, EVG=elvitegravir, DTG=dolutegravir, qd=once daily,

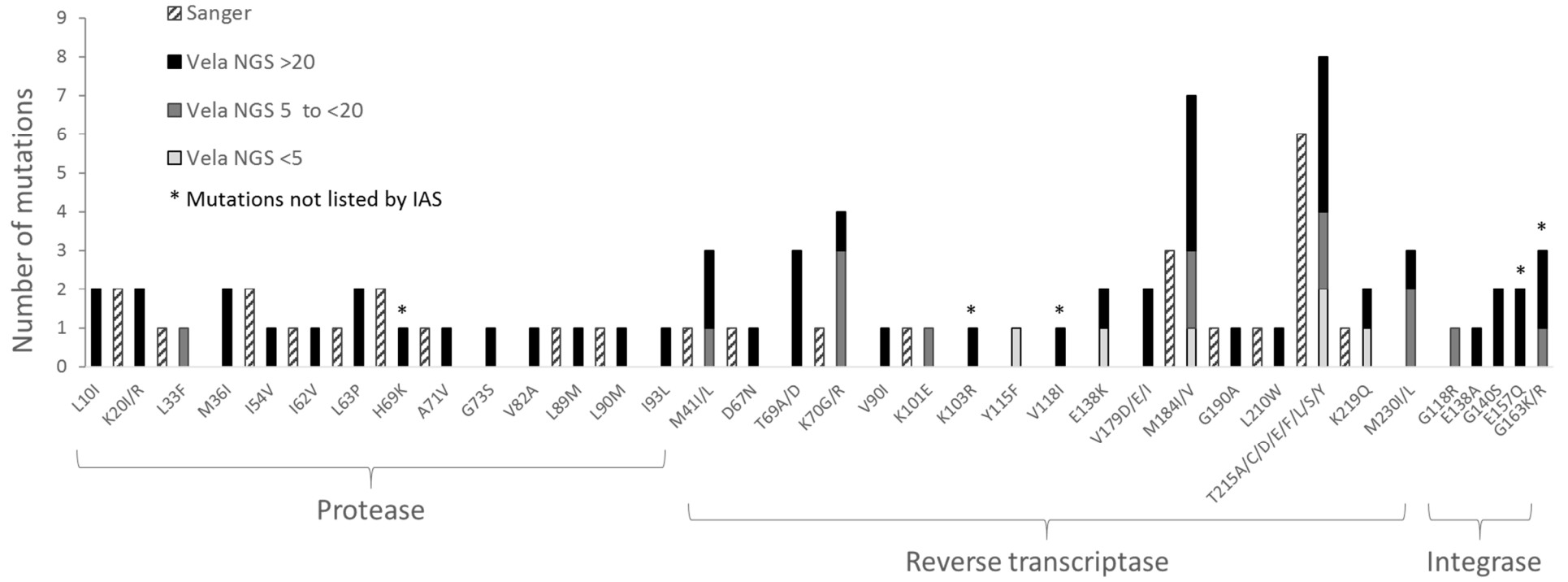
333 BIC=bictegravir, CAB=cabotegravir; ^cTDF=tenofovir, FTC=emtricitabine, DRV/r=ritonavir-boosted darunavir, 3TC=lamivudine, RPV=rilpivirine, ATV/r=ritonavir-

334 boosted atazanavir, EFV=efavirenz

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Figure 1



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