Clinical impact of Ultra-Deep Versus Sanger Sequencing detection of minority mutations on the HIV-1 Drug Resistance interpretations after virological failure

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Aims

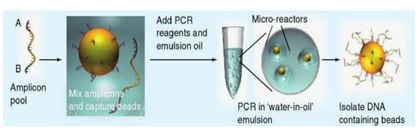
The study had 3 aims, comparison of Sanger sequencing and Ultra-deep sequencing (UDS); the comparison of the three most commonly used HIV drug resistance interpretation algorithms and the comparison of resistance interpretation of UDS data at two viral detection thresholds (1% and 20%).

Introduction

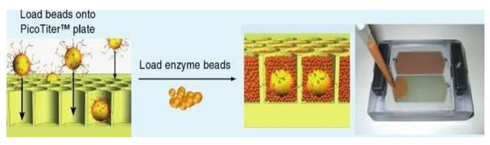
Drug resistance mutations are routinely detected using standard Sanger sequencing, which does not detect minor variants with a frequency below 20%. Several studies have clearly demonstrated that patients with mutation rates between 1% and 20%, particularly for NNRTIs, are more likely to fail therapy. It is therefore important to detect minor variants that occur below 20% frequency using a robust and sensitive method available at an affordable price and providing an easier interpretation for HIV drug resistance (DR) monitoring. The impact of detecting minor variants generated by UDS on HIV DR interpretations has not yet been studied.

Methods

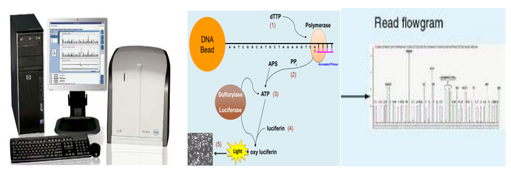
Fifty HIV-1 patients who experienced virological failure were included in this retrospective study. The HIV-1 UDS protocol allowed the detection and quantification of HIV-1 protease and reverse transcriptase variants related to genotypes A, B, C, F and G. DeepChek®-HIV simplified DR interpretation software was used to compare Sanger sequencing and UDS.



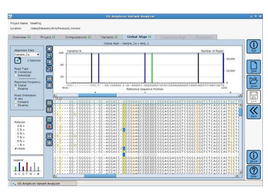
A) Pooled amplicons are clonally amplified in droplet emulsions.



B) Isolated DNA-carrying beads are loaded into individual wells on a PicoTiter™ plate and surrounded by enzyme beads.



C) Nucleotides are flowed one at a time over the plate and template-dependent incorporation releases pyrophosphate, which is converted to light through an enzymatic process. The light, which are proportional to the number of incorporated nucleotides in a given flow, are represented in flowgrams .



D) nucleotide sequence is determined for each read with the GS Amplicon Variant Analyzer software (Roche).

E) Clinical genotyping report using DeepChek expert system and clinical utilities – CE-IVD marked (ABL, SA and TherapyEdgeTM157 , USA)

Results

The total time required for the UDS protocol was found to be approximately three times longer than Sanger sequencing with equivalent reagent costs. UDS detected all of the mutations found by population sequencing and identified additional resistance variants in all patients. An analysis of DR revealed a total of 643 and 224 clinically relevant mutations by UDS and Sanger sequencing, respectively. A significant difference in the DR interpretations for 19 antiretroviral drugs was observed between the UDS and Sanger sequencing methods. Y181C and T215Y were the most frequent mutations associated with interpretation differences.

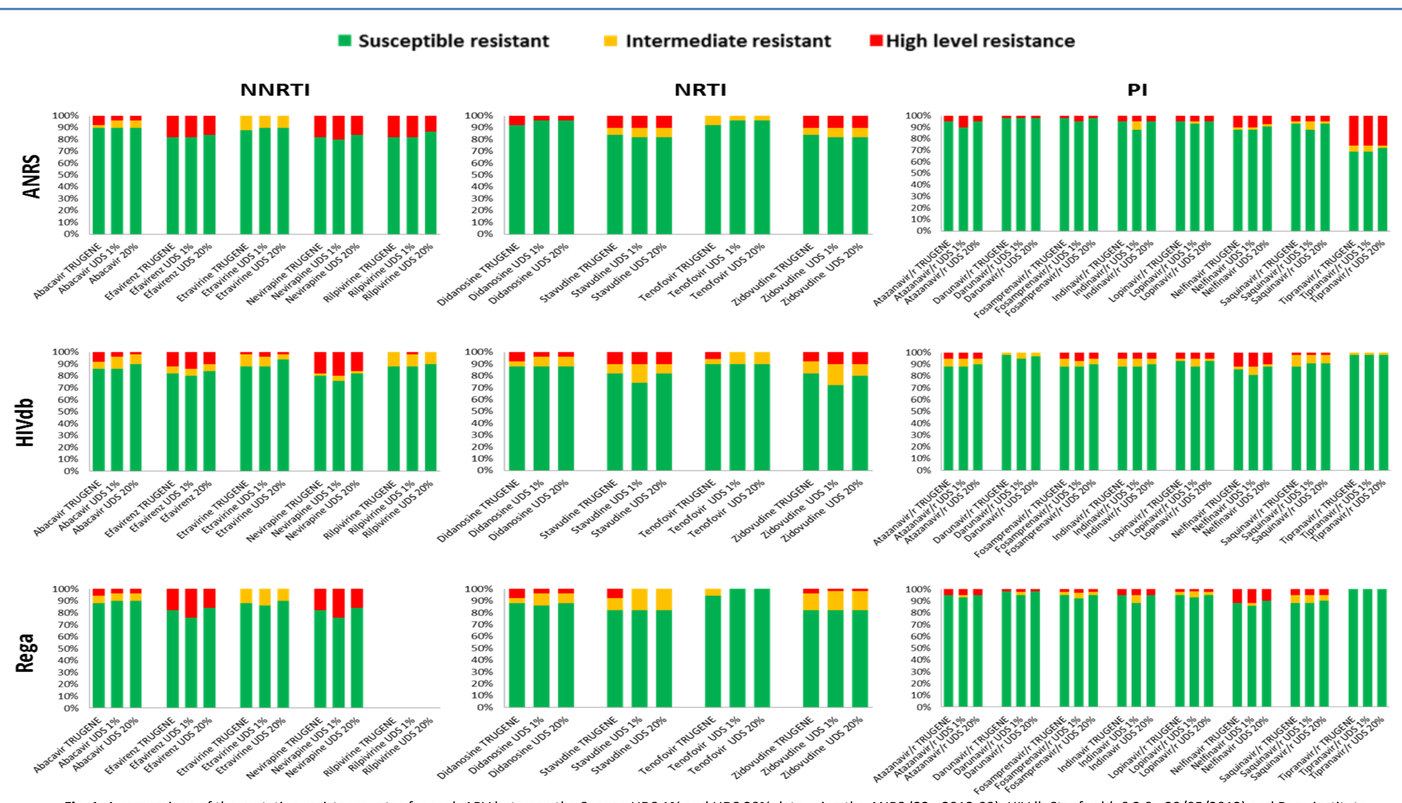


Fig. 1. A comparison of the mutation resistance rates for each ARV between the Sanger, UDS 1% and UDS 20% data using the ANRS (22 - 2012-09), HIVdb Stanford (v6.2.0 - 29/05/2012) and Rega institute (v8.0.2 - 16/06/2009) algorithms on 50 HIV-infected patients. Green (S), yellow (I) and red (R).

Table 1. An analysis of the major discrepant results using the ANRS, HIVdb and Rega rules-based algorithms.

Drug	Algorithms	Mutations (rules)[a]	Resistance interpretation
Efavirenz	ANRS	**Y181C**	Resistance
	HIVdb	**Y181C**	two-fold ↘ susceptibility
	Rega	**Y181C**, **M230I**, **G190E**	Resistance
Etravirine	ANRS	Combination of four mutations	Resistance
	HIVdb	**G190E**	Intermediate-to-high level resistance
	Rega	Combination of three mutations	Resistance
Rilpivirine	ANRS	**Y181C**	Associated with resistance
	HIVdb	**Y181C**	three-fold ↘ susceptibility
	Rega	No mutation	No interpretation
Didanosine	ANRS	K65R	Intermediate
	HIVdb	**T215Y**	Low level resistance
	Rega	At least one mutation of 65NR, 70EG, 74IV, 75T or a combination of two or three mutations	Intermediate
Stavudine	ANRS	**T215Y**	Resistance
	HIVdb	**T215Y**	Resistance
	Rega[b]	Combination of three or four mutations	Resistance
Tenovofir	ANRS	Combination of three, four or five mutations	Intermediate
	HIVdb	**T215Y**	Low-level resistance
	Rega	70EG or combination of two, three or four mutations	Intermediate
Zidovudine	ANRS	**T215Y**	Resistance
	HIVdb	**T215Y**	Resistance
	Rega	151M or combination of three or four mutations	Resistance
Darunavir	ANRS	At least four mutations	Resistance
	HIVdb	No mutations are associated with the highest levels	–
	Rega	GSS > 3.5	Resistance
Tipranavir/r	ANRS	GSS > 3 :**36I**/L/V –53L/W/Y + 58E **+ 69I/K**/N/Q/R/Y + **89I/M**/R/T/V	Resistance
	HIVdb	M46IL	↘ Susceptibility with other resistance mutations
	Rega	combination to obtain score at least 3.5	Resistance

[a]Mutation or combination of mutations. Lists of mutation combinations associated with resistance for the ANRS, HIVdb and Rega algorithms are accessible online through the Stanford database website (http://hivdb.stanford.edu/).
[b]The T215 mutation has been identified as a key mutation in a pathway leading to high-level resistance.
GSS, Genotypic susceptibility score; ↘, reduced. Mutations in bold were responsible for the DR interpretation differences.

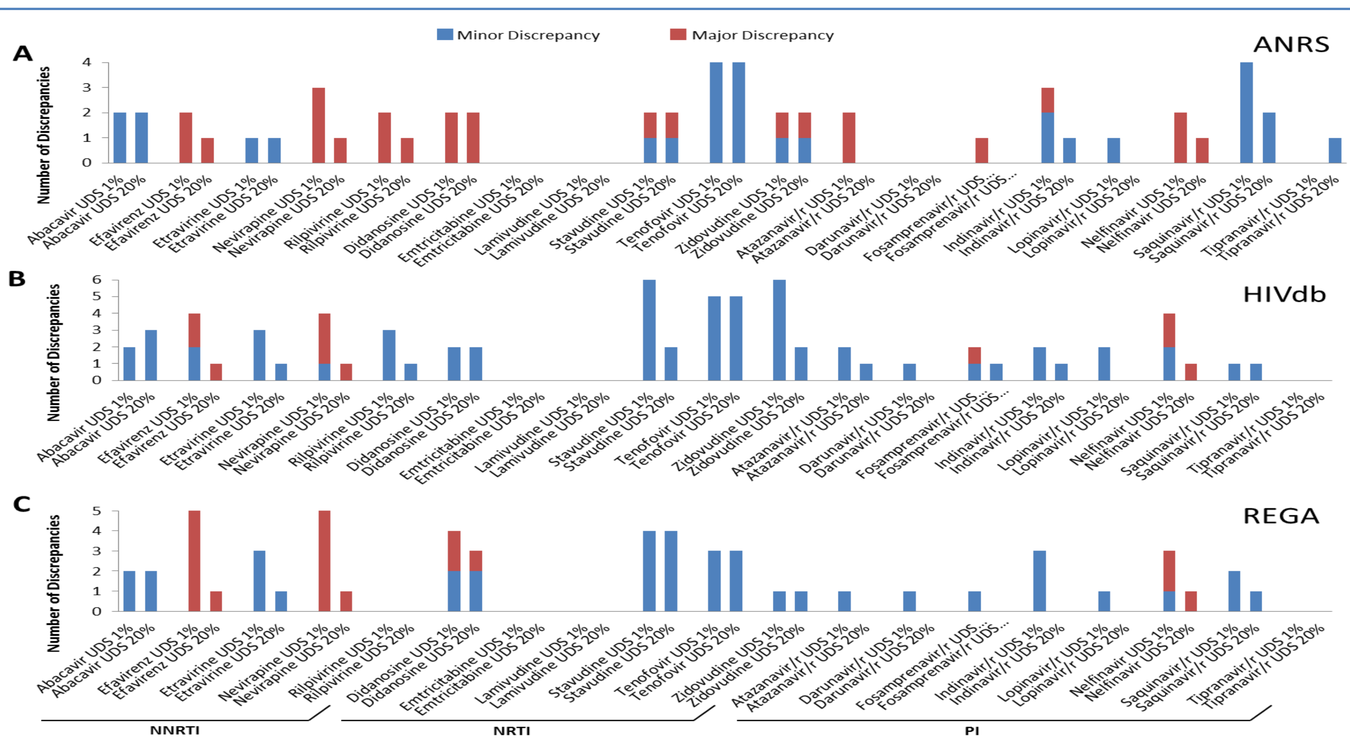


Fig. 2. The number of minor and major discrepancies for each ARV between UDS (>1% and ≥20%) and Sanger sequencing using the ANRS, HIVdb and Rega algorithms. (A) ANRS (22 - 2012-09), (B) Rega institute (v8.0.2 - 16/06/2009), (C) HIVdb Stanford (v6.2.0 - 29/05/2012). **Minor discrepancy** corresponded to having an intermediate interpretation in one algorithm, with a susceptible or resistant result in the other. (I → R or S). **Major discrepancy** was defined as having a susceptible result from one algorithm and resistant from the other (S → R).

Table 2. A comparison of the major and minor discrepancies identified between the Sanger sequencing and UDS methods according to the threshold selected.

	Algorithm	Minor Discrepancy	Major Discrepancy
Sanger vs. UDS 1%	ANRS	1.68% (16/950[a])	1.79% (17/950)
Sanger vs. UDS 20%		1.37% (13/950)	0.84% (8/950)
P-value		0.29	0.035
Sanger vs. UDS 1%	HIVdb	4.32% (41/950)	0.84% (8/950)
Sanger vs. UDS 20%		2.11% (20/950)	0.32% (3/950)
P-value		0.003	0.068
Sanger vs. UDS 1%	REGA	2.57% (27/1050[b])	1.81% (19/1050)
Sanger vs. UDS 20%		1.33% (14/1050)	0.48% (5/1050)
P-value		0.020	0.002

[a]950 is the number of calculation tests between the 19 ARV and 50 patients (ANRS and HIVdb give not data for Atazanavir and delarvirdine).[b]1050 is the number of calculation tests between the 21 ARV and 50 patients (The Rega algorithm gave an interpretation of resistance for Atazanavir and delarvirdine).
P-values were calculated using a Chi-Square test performed with SAS V9.1 software (SAS Institute Inc., Cary, NC, USA).

Conclusion

We have clearly shown that the numbers of mutations detected using UDS 1%, UDS 20% and Sanger sequencing are significantly different. When compared with the Sanger method, the UDS 1% data showed more minor and major discrepancies than the UDS 20% data. A combination of UDS and DeepChek® software for the interpretation of DR results would help clinicians provide suitable treatments. A cut-off of 1% allowed a better characterisation of the viral population by identifying additional resistance mutations and improving the DR interpretation.