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Depth coverage and HIV-1 variability negatively affect the performance of the "HIV-1 solution v2" NGS sequencing kit for drug resistance monitoring

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Introduction/Summary

- The "HIV-1 solution v2" kit (Arrow Diagnostics) is an In Vitro Diagnostic (IVD) certified next-generation sequencing (NGS) system that has recently become available for routine HIV-1 drug resistance genotyping.
- A previous study has shown that this system can reliably identify resistance mutations with frequency >10%, although suboptimal sequence coverage can compromise the sensitivity[1].
- This study aims to investigate the prevalence and the factors contributing to reduced sequence coverage by analyzing NGS data obtained from different Italian laboratories.

Methods

- Routine NGS data from viral RNA generated through the "HIV-1 Solution v2" kit on Illumina MiSeq or iSeq100 platforms were collected with the relative viral load from four Italian laboratories.
- For the MiSeq instrument, fastQs were considered acceptable if the cluster density of the relative run was >600 and >800 K/mm^2 when using Nano and Standard 2x250 bp v2 reagents, respectively.
- Median read depth and sequence coverage were analyzed through the HIVdb-NGS (beta) program (HIVdb Stanford) using ≥100 minimum read depth, while viral subtype on consensus sequences for each sample was determined through the COMET HIV-1 tool.

Results

- We collected 343 fastQs, 302 (88%) and 41 (12%) were generated through the MiSeq and iSeq100 platforms, respectively, while 216 and 86 were obtained using Standard and Nano reagent kits for MiSeq, respectively. Samples had a median viral load of 4.92 [IQR 4.22-5.41] log HIV-1 RNA copies/mL.

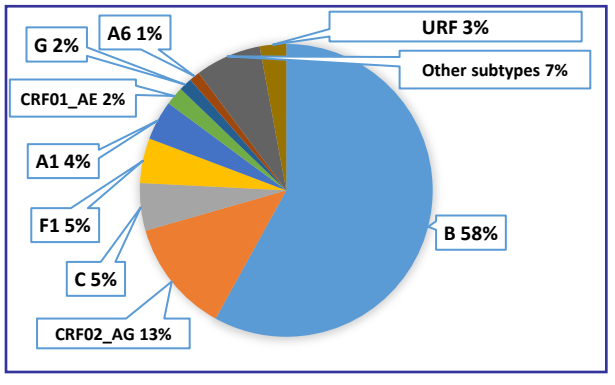

Figure 1. Distribution of viral subtypes among consensus sequences (n=343)

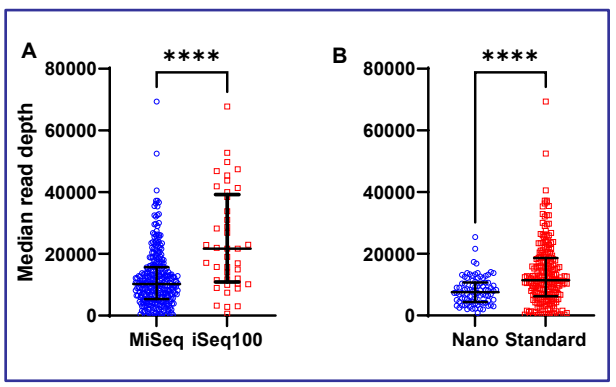

Figure 2. Distribution of median read depth values obtained with (A) the MiSeq or iSeq100 instrument or (B) the Nano or Standard reagent kit. **** = p<0.0001

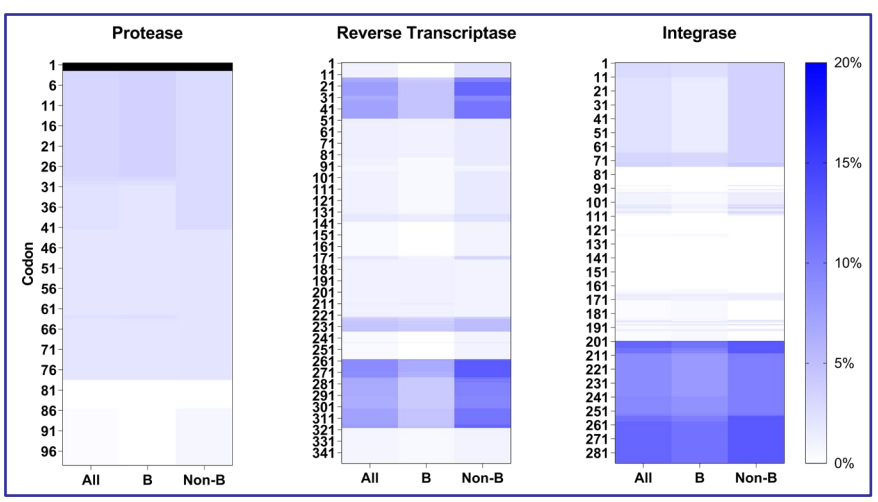

Figure 3. Heatmap of the frequency of regions with low coverage (<100 reads/position) within protease, reverse transcriptase and integrase sequences with according to the viral subtype (B vs. non-B)

Table 1. Analysis of the predictors of low coverage (regions with <100 reads/position)

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Subtype (non-B vs. B)	1.90	1.16-3.13	**0.011**	1.87	1.14-3.06	**0.013**
Instrument (MiSeq vs. iSeq100)	0.50	0.16-1.60	0.245			
Viral load	0.97	0.74-1.28	0.852			
Median read depth	0.25	0.14-0.45	**<0.0001**	0.24	0.14-0.42	**<0.0001**
Nano vs Standard reagent kit	0.97	0.71-2.21	0.437			

- Subtype B was identified in 199 cases (58%), while the most prevalent non-B subtypes were CRF02_AG (n=43, 12.5%), C (n=18, 5.0%) and F1 (n=17, 5.0%) (***Figure 1***).
- Higher median read depth was obtained with iSeq100 platform (21684 [IQR 10932-39250] vs. 10241 [5370-15705] with MiSeq, p<0.0001, Mann-Whitney test) and with Standard (11449 [6223-18616] vs. 7554 [4397-10709] with Nano reagent kit, p<0.0001, Mann-Whitney test) (***Figure 2***).
- Globally, low coverage issues (regions with <100 reads/position) were detected in 101 (29.4%) sequences, mostly affecting coding regions within reverse transcriptase (RT) than integrase (IN) and protease (68, 52 and 12 cases, respectively). In particular, the most affected regions were at codons 14-49 (frequency 5% B vs. 11% non-B), 225-235 (4.4% B vs. 5.6% non-B) and 260-319 (5.3% B vs. 11.1% non-B) of RT, codons 201-288 (9.5% B vs. 11.7 non-B) of IN (***Figure 3***). Of note, all regions affected by low coverage include at least one position associated with resistance to antiretrovirals. Regions with low coverage were mostly associated with non-B subtypes (55/144 cases [38.2%] vs. 46/199 [23.1%] in subtype B) and with MiSeq instrument (94/302 cases [31.1%] vs. 6/41 [14.6%] with iSeq100), while no difference was observed when considering Nano vs. Standard reagent kit.
- After correcting for viral load and the type of instrument, non-B subtype and median read depth were identified as independent predictors of low coverage by multivariate analysis (p=0.013 and p<0.0001, respectively) (***Table 1***).

Conclusions

- This analysis on real life settings suggests that HIV-1 variability and depth of sequence coverage can affect the performance of the "HIV-1 solution v2" kit, confirming previous findings.
- Further upgrades of this sequencing kit are needed to improve the sequence coverage of non-B subtypes and the read depth among different sequencing platforms.

Reference

1. Armenia et al. Comparison of different interpretation tools for HIV-1 resistance detected through Next Generation Sequencing in Italian clinical routine. Oral communication OC129,15th Italian Conference on AIDS and Antiviral Research, Bari (Italy, June 14-16, 2023); oral communication, abstract n.8, European Meeting on HIV & Hepatitis, Rome (Italy, June 7-9, 2023).