Implications of Next-Generation Sequencing for Drug Resistance and Cluster Detection

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Poster ID 0932

Background

HIV-1 polymerase (pol) sequences from routine HIV drug resistance testing are used to monitor drug resistance and identify molecular transmission clusters as part of public health surveillance. Proviral DNA drug resistance testing using next-generation sequencing (DNA-NGS) has been used clinically since 2015 to provide drug resistance information in the setting of viral suppression. Since DNA-NGS covers the same part of the HIV genome and DNA-NGS consensus sequences mimic traditional RNA-Sanger (RNA-S) sequences, they have likely been reported to public health as RNA-S sequences (Figure 1). Some clinical labs are also using (and others are considering) NGS for RNA-based drug resistance testing (RNA-NGS). We evaluated whether shifts in testing methods and sequencing technology have implications for public health surveillance of drug resistance and transmission clusters.

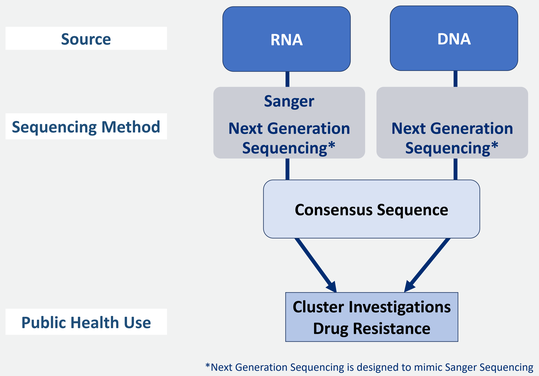
Purpose of the Study:

To explore the implications of resistance testing using next-generation sequencing and DNA-proviral sequencing for public health monitoring of drug resistance and clustering.

Methods

We identified 114,866 RNA-S, 10,624 DNA-NGS, and 4,535 RNA-NGS sequences reported to New York during 2010-2019 (Figure 2). Inferred drug resistance was compared for 1,350 persons with two or more sequence types. For cluster analyses, pairwise genetic distances were calculated between sequences for the same person with collection dates within 1 year (n=7,771 comparisons from 2,823 individuals) using Secure HIV-TRACE default settings and a 2% genetic distance threshold, stratified by sequence type.

Figure 1: HIV Nucleotide Sequence Reporting to Public Health Labs



*Next Generation Sequencing is designed to mimic Sanger Sequencing

Results

Figure 2: HIV-1 Drug Resistance Test Type by Year-Quarter

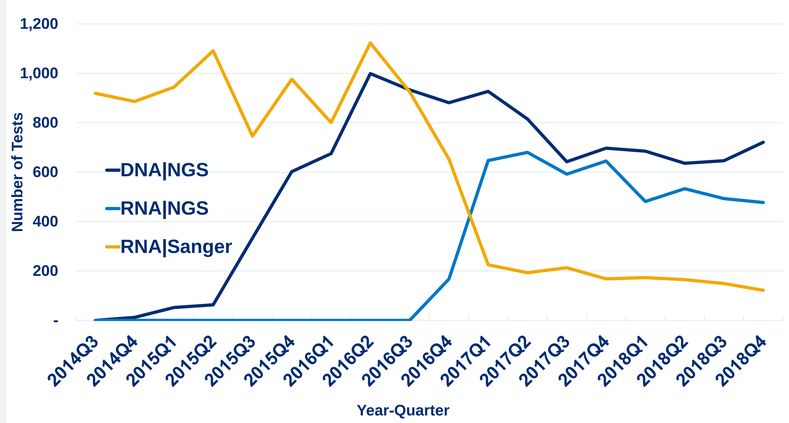
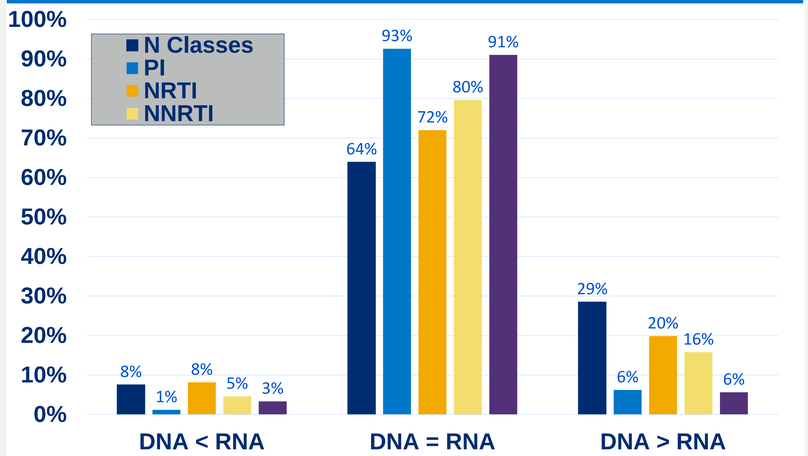


Figure 3: Within-Individual Inferred Drug Resistance, DNA vs RNA Sequences



N Classes = Number of drug classes showing intermediate or high resistance in at least one drug in that class; PI = Protease Inhibitor; NRTI = Nucleoside Reverse Transcriptase Inhibitor; NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitor; IN = Integrase Inhibitor

Overall, drug resistance was more likely to be inferred from DNA-NGS sequences than RNA-based sequences from the same individual (29% of comparisons vs. 8% where the RNA sequence showed higher resistance) and individual drug classes showed similar results (Figure 3). RNA-S and RNA-NGS were combined for this analysis because their resistance results were similar. Time between tests was not a significant factor (not shown).

For clustering, over 25% of DNA-NGS were rejected by Secure HIV-TRACE due to high levels of ambiguities compared to RNA-NGS (11%) and RNA-S (8%). Based on pairwise distances for sequences from the same individual, RNA-NGS and especially DNA-NGS sequences, clustered less frequently than RNA-S sequences and at a higher distance threshold if they did cluster. Mean number of years since diagnosis was over 13 years and varied by sequence type but did not explain the results (Table 1).

Table 1: Within-Individual Pairwise TN93 Distances by Sequence Type[1]

Sequence Comparison	N	% clustering at <1.5% distance level[2]	% clustering at <0.5% distance level[3]	Mean Years since Dx
DNA-NGS to DNA-NGS	229	60.7	19.2	16.8
DNA-NGS to RNA-NGS	121	67.8	25.6	13.5
DNA-NGS to RNA-S	857	69.7	23.0	14.7
RNA-NGS to RNA-NGS	262	69.8	16.4	16.8
RNA-NGS to RNA-S	707	75.7	27.3	14.8
RNA-S to RNA-S	5,595	79.0	30.6	19.1

[1] Comparisons are within an individual from *pol* sequences collected < 1 year apart
[2] 1.5% distance threshold is the Secure HIV-TRACE default
[3] Clustering at the 0.5% distance threshold indicates recent and rapid transmission per CDC

Conclusions

We found significant differences between consensus DNA-NGS and RNA-NGS sequences compared to RNA-S sequences for cluster inference and between DNA-NGS and RNA-based sequences for drug resistance. Hence, reporting of sequence type for public health surveillance is critical for ensuring appropriate inclusion of sequences for accurate HIV drug resistance, transmission cluster analyses and interpretation of data. Monitoring changes in sequencing technology is critical for assessing its impact on public health and clinical decisions.

Limitations

The single lab producing these NGS sequences intentionally configures their NGS pipeline's threshold for calling ambiguities to mimic Sanger sequencing output. NGS sequences from different labs or generated using different pipeline settings may show different results when compared to Sanger sequences. Lastly, in almost all cases we didn't have sequences of different types taken from the same individual on the same day because this study relied on existing reported sequences.

References

1. Kosakovsky Pond SL, Weaver S, Leigh Brown AJ, Wertheim JO (2018) HIV-TRACE (Transmission Cluster Engine): a tool for large scale molecular epidemiology of HIV-1 and other rapidly evolving pathogens Mol Biol Evol.

This analysis was made possible with the support of the Centers for Disease Control and Prevention PS18-1802: Integrated Human Immunodeficiency Virus (HIV) Surveillance and Prevention Programs for Health Departments.
The authors acknowledge the contribution of colleagues in the Bureau of HIV/AIDS Epidemiology to this analysis.
The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.