The Cross-Subtype Intact Proviral DNA Assay Detects >97% of Proviral Sequences from Diverse HIV Clades

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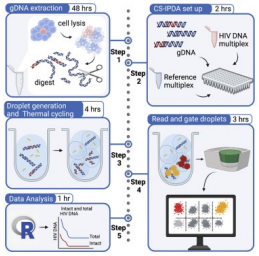
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Introduction

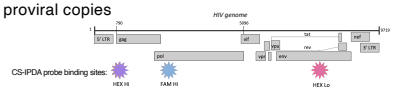
- Intact proviral DNA assays (IPDA) are high-throughput methods to quantify intact and defective HIV proviruses.
- IPDAs provide estimates of rebound-competent HIV reservoir during suppressive ART.
- Most IPDAs aredesigned for subtype B and can have up to ~28% false negative rate due to polymorphisms in the primer/probe sites, even in subtype B samples[1]
- The cross-subtype IPDA (CS-IPDA) was designed to tolerate genetic variation across subtypes A, B, C, D and CRF01_AE,[2] and here we utilized on Kenyan pediatric cohorts.

Methods

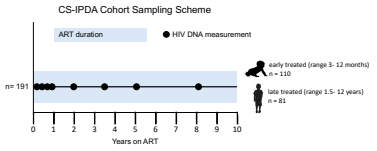
- The Cross Subtype Intact Proviral DNA Assay (CS-IPDA) is a multiplexed digital droplet PCR[2,3]



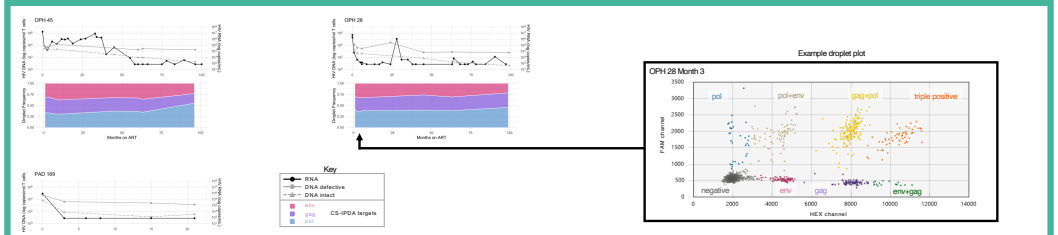
- Three HIV genomic regions are probed (*gag, pol,* and *env*) to quantify intact and defective proviruses
- Triple-positive droplets estimate intact provirus copies
- Single- and dual-positive droplets estimate defective proviral copies



- We applied the CS-IPDA to PBMCs collected during ART from 191 children living with HIV in Kenya, where subtypes A, C, and D are prevalent.
- Samples were tested in ≥3 replicates and included if >1e5 cells were tested (median: 4.2e5, range:1e5 - 4.3e6) and if shearing index was <40% (median 3%, range 2% - 31%).

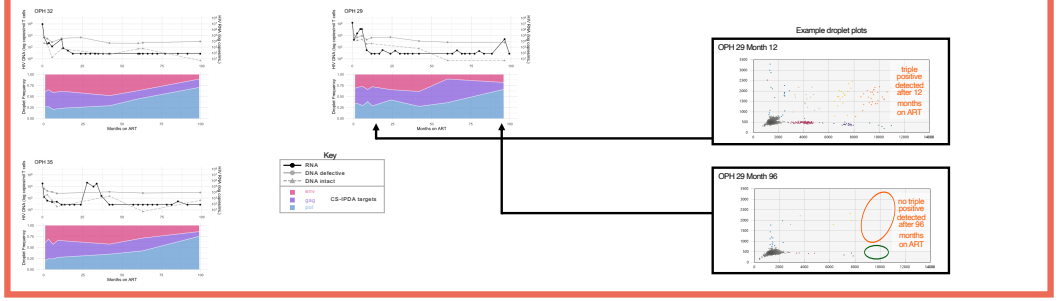


Examples with detectable intact provirus and all three genomic regions (86.9%, 605/696 samples)

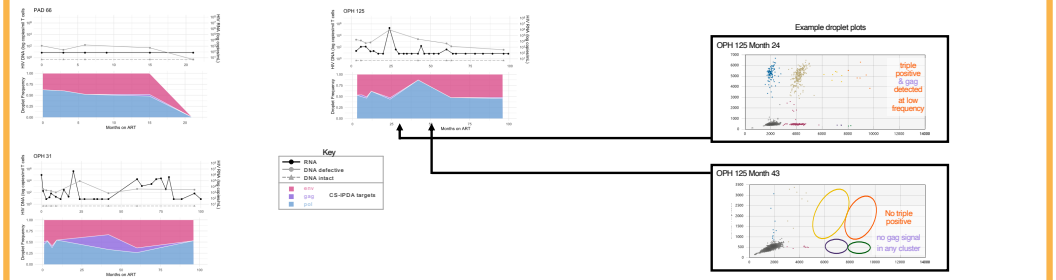


Representative examples of longitudinal data shown. **Top graphs**: HIV DNA quantified by CS-IPDA in grey (defective HIV DNA shown in circles/solid lines and intact HIV DNA shown in triangles/dotted lines) and HIV RNA quantified by Gen-Probe in black (circles/solid lines). Samples below the LLOD were set to half the LLOD (1 copy/ml T cells for DNA and 150 copies/mL for RNA) and zero. **Bottom plots**: Relative frequency of each of the three CS-IPDA targets (env in pink, gag in purple, pol in blue), calculated as the fraction of droplets positive for each target out of the total number of positive droplets. **Examples of Droplet plots**: example droplet plot from QuantaSoft Analysis Pro. The env and gag targets are multiplexed in the HEX channel, while pol is in the FAM channel. Single- double- and triple-positive droplet clusters have been circled and labeled for illustrative purposes. Time points from specific participants expanded in the example droplet plots are indicated by an arrow.

Examples with no detectable intact provirus, but all three genomic regions were detected (10.7%, 75/696)



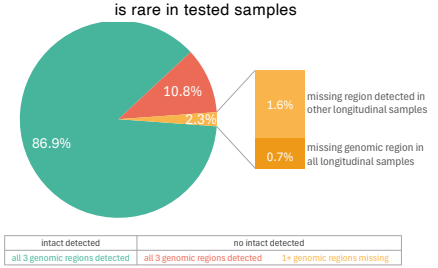
Examples with no detectable intact provirus and missing genomic region(s) (2.3%, 16/696)



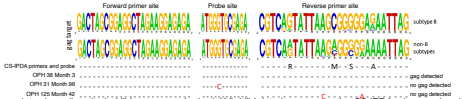
1. Kinloch, NN, et al. (2021) HIV-1 diversity considerations in the application of the Intact Proviral DNA Assay (IPDA). Nat Commun *12*, 165.
2. Cassidy, NAJ, et al. (2022) HIV reservoir quantification using cross-subtype multiplex ddPCR. iScience *25*, 103615.
3. FISH, CS, et. al. (2022) Protocol for high-throughput reservoir quantification across global HIV subtypes using a cross-subtype intact proviral DNA assay. STAR Protoc *3*, 101681.

Results

Failure to detect one of the CS-IPDA targets is rare in tested samples



- Across 696 samples collected longitudinally from 191 ART-treated children, there was median 1.4e3 defective (range: 0 – 6.8e4) and 145 intact (range: 0 – 5.2e4) HIV DNA copies per million T cells.
- In 91 (13.1%) samples, no intact proviruses were detected, which could indicate intact levels truly below detection or false negatives due to primer/probe mismatches.
- In 75 (10.7%) samples, intact HIV DNA was undetectable - but all 3 genomic targets were detected in the defective proviral population (in single- or dual-positive droplets), suggesting the negative intact result was not due to polymorphisms causing failure of the PCR to detect a region of the provirus.
- In only 16 (2.3%) of the 696 samples tested, the CS-IPDA failed to detect any signal for one of the targets, likely due to polymorphisms in the primers and/or probe binding sites.
- illumina sequencing of the gag region found polymorphisms in the probe or primer binding sites from samples with no gag detected.



Logo plots show diversity in HIV sequences in the LANL HIV data base across the CS-IPDA primer and probe binding sites for the gag target for subtype B and non-subtype B sequences. Changes in the CS-IPDA primer sequence to account for diversity are shown in the first row. Examples of sequenced participant samples are shown below, with mismatches to the CS-IPDA sequence shown in red and matches shown as dashes.

Conclusions

The CS-IPDA has a very low false-negative rate due to PCR failure in samples from Kenya, where the most prevalent global subtypes circulate, suggesting it effectively tolerates diversity across multiple subtypes

Acknowledgements

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