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HIV Diversity Considerations in the Application of the Intact Proviral DNA Assay (IPDA)

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none to declare

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BACKGROUND and OBJECTIVE:

The Intact Proviral DNA Assay (IPDA)[1] was developed to address the need for a scalable, selective assay to quantify genomically intact proviruses in the HIV reservoir. The IPDA is a duplexed droplet digital PCR assay targeting two regions of HIV likely to be present in intact proviruses: the packaging signal (Ψ) and Rev Responsive Element (RRE) in *env*. Results of the IPDA have previously been shown to correlate with the field gold standard for intact reservoir quantification, QVOA, and the assay is rapidly being adopted in research studies[2,3] and as an endpoint in clinical trials[4]. However, the widespread applicability of the IPDA to diverse cohorts is unknown. We applied the IPDA to our cohort of HIV Subtype B- infected individuals from across North America to address this question.

COHORT, METHODS and RESULTS (Figure 1):

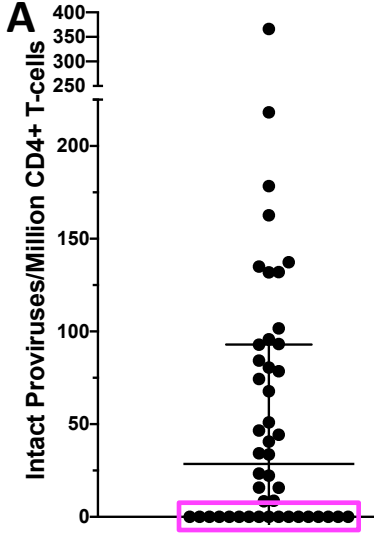
Cohort:

- N= 46 (N= 15 Vancouver, N= 19 Toronto, N= 4 New York, N= 7 Washington DC, N=1 Mexico City)
- ARV-suppressed
- Subtype B-infected
- pre-ART infection duration:
  - 1 month to >10 years
- QVOA measurements also available for 37 participants

Methods:

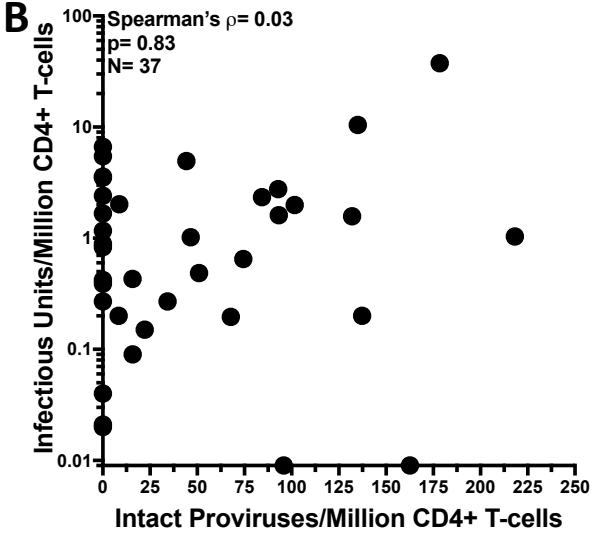
- CD4+ T-cells were isolated from PBMCs by negative selection, following which genomic DNA was extracted
- the IPDA was then preformed as previously described[1]

Results (Figure 1): Application of the IPDA to a diverse North American cohort



We observed a median 29 (IQR 0- 93) Intact Proviruses/ Million CD4+ T-cells in our cohort (**Figure 1A, left**).

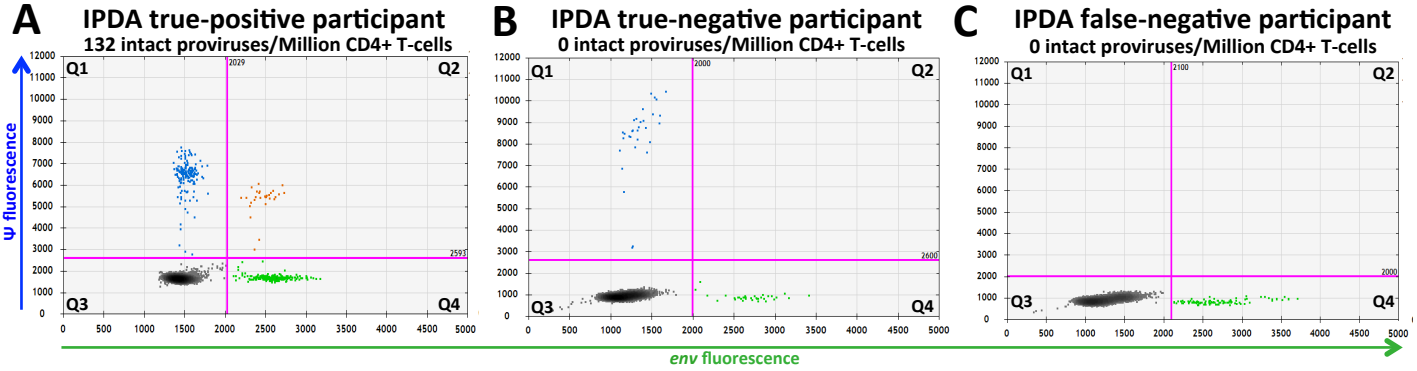
However, we did <u>not</u> observe a correlation between Intact Proviruses/ Million CD4+ T-cells (IPDA) and Infectious Units/Million CD4+ T-cells (QVOA) (Spearman's ρ= 0.03, p= 0.83) (**1B, right**).



Curiously, in 17/47 participants, intact proviruses <u>could not be detected</u> (1A, pink box), despite recovery of replication competent virus by QVOA in 15/15 individuals with an available measurement.

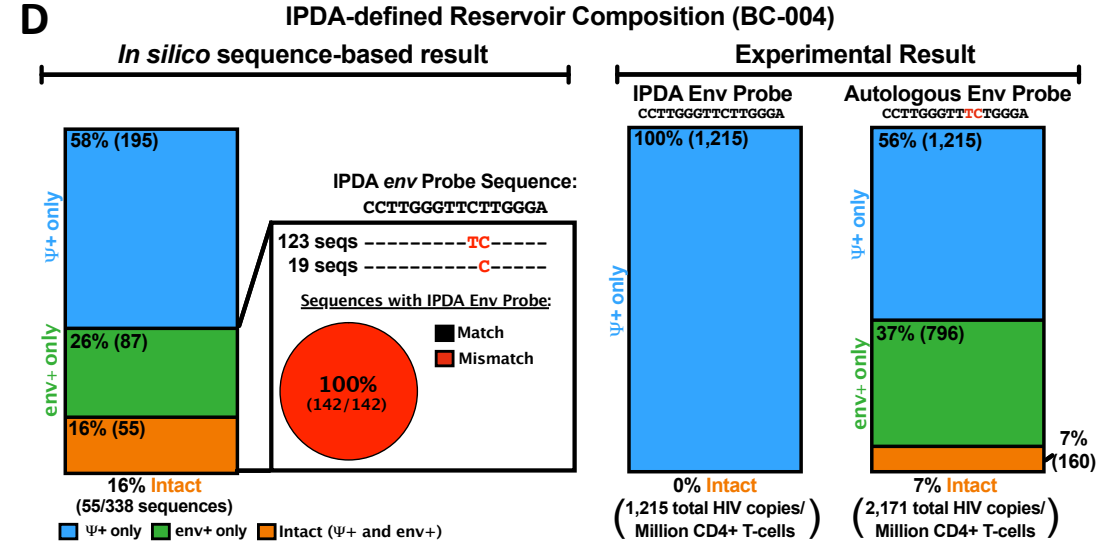
[1]Bruner et al. PMID: 30700913; [2]Antar et al. PMID: 32191639; [3]Peluso et al. PMID: 32045386; [4]Wilkin & Jones, Personal Comm. 2019

RESULTS (Figure 2): Inter-individual HIV diversity can lead to false-negative IPDA results



In IPDA true-positive participants, Ψ- and *env*- single positive, as well as intact (Ψ- and *env*-double positive) proviruses are detectable (see **Figure 2A,** droplets in Q1, Q4 and Q2 respectively). In 4/17 IPDA-negative participants, both Ψ- and *env*-single positive proviruses, but no intact proviruses, were detected, suggesting a true negative result (**Figure 2B**).

However, in 13/17 individuals, only Ψ- or *env*- single positive proviruses, or no proviruses, were detected (**Figure 2C,** no droplets in Q1 and Q2). Specifically, in 8 individuals only Ψ-positive proviruses could be detected (*env*-negative), in 4 only *env*-positive proviruses (Ψ- negative) could be detected and in one participant no proviruses could be detected. **This suggests a false-negative result.**



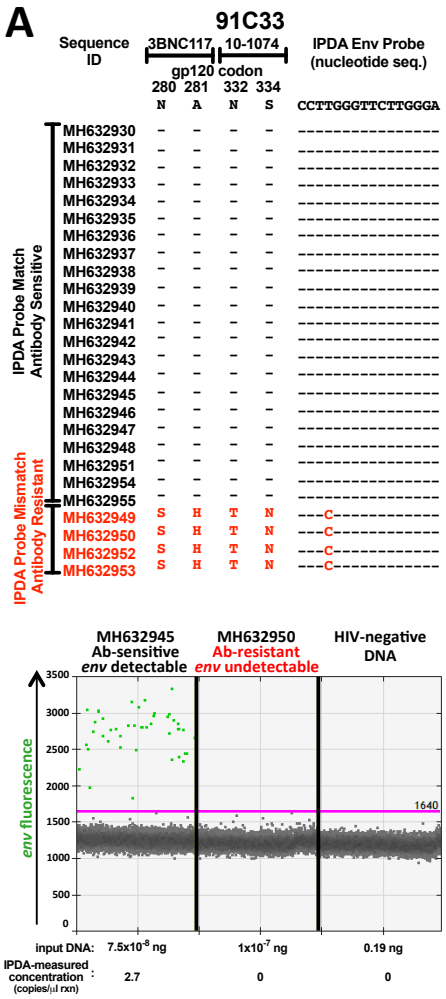
Single-genome, near-full-length proviral sequencing of IPDA *env*-negative participant BC-004 revealed mismatches to the *env* probe. *In silico* predicted reservoir distributions taking these polymorphisms into account (**2D, left**) differed markedly from the experimentally obtained result (**2D, centre**).

Substitution of an autologous *env* probe rescued detection to *in silico*-predicted levels (**2D, right**), confirming that HIV polymorphism can cause the IPDA to fail.

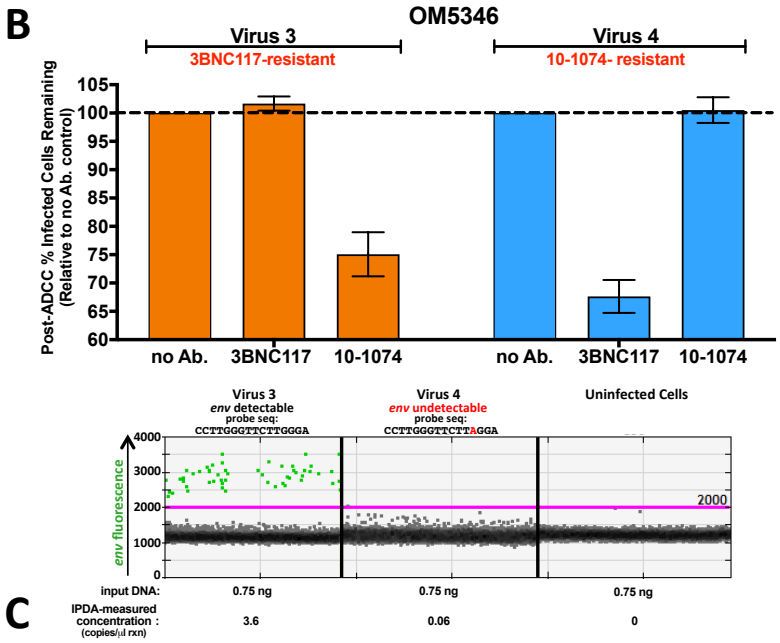
HIV sequencing of all other cases of presumed assay failure revealed mismatches in the probe and/or 3' end of a primer.

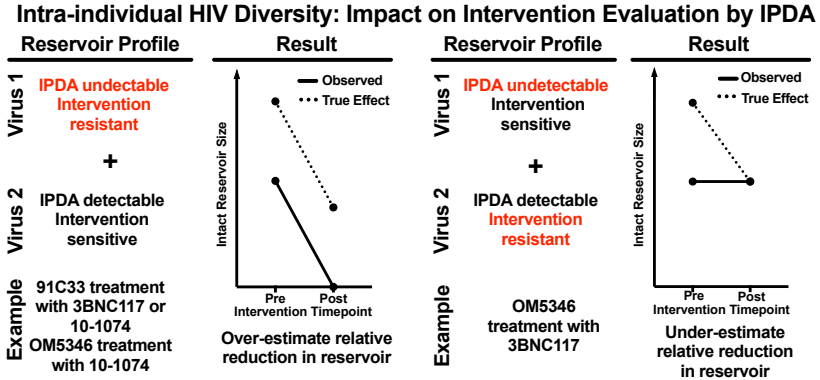
This yields an overall estimated false-negative rate of 13/46 (28%)

RESULTS (Figure 3): Intra-individual diversity can lead to reservoir underestimation by IPDA



[5]Bar-On et al. PMID: 30258217





Within-host HIV diversity could lead to underestimation of intact reservoir size if IPDA- detectable and undetectable sequences co-exist.

This is not easy to identify and is especially concerning if these HIV sub-populations also differ in susceptibility to cure interventions, such as broadly neutralizing antibodies (bNAbs). If so, erroneous conclusions could be drawn about the intervention's efficacy if IPDA is used as a trial readout.

Individuals 91C33 (published literature[5]) and OM5346 (our cohort) provide hypothetical examples:

91C33 did not respond to off-ART infusions[5] of bNAbs 3BNC117 and 10-1074 because they harboured a plasma HIV sub-population resistant to both antibodies (**Figure 3A, top**). This same population also harboured a mismatch to the IPDA *env* probe (**3A, top**), which we experimentally confirm cannot be detected by the IPDA (**3A, bottom**).

Therefore, should a person harbouring such diversity in their reservoir be successfully treated with these bNAbs, the IPDA would over-estimate the intervention's effect (**3C, left**).

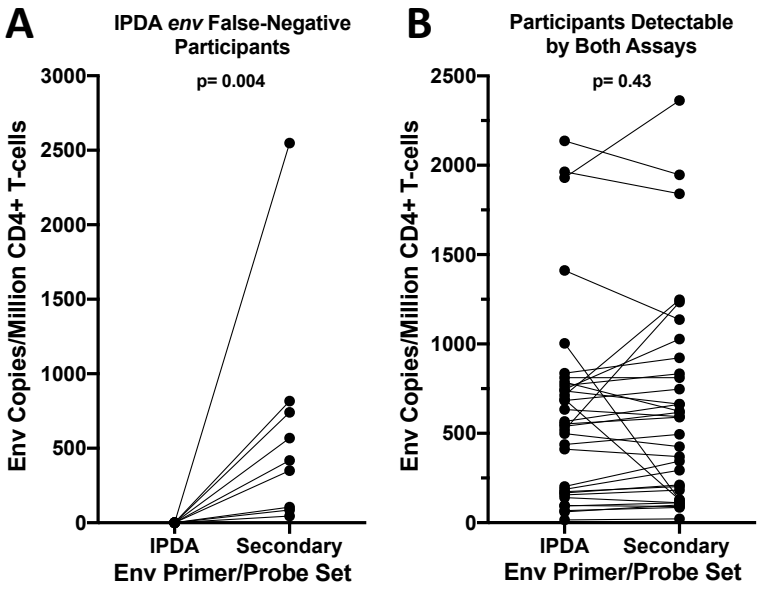
OM5346 is co-infected with both a subtype B and a non-B strain and harbors replication competent viruses of both strains (subtype B= 'virus 3', non-B= 'virus 4')(**3B, top**).

Cells infected with these viruses harbour opposing sensitivities to 3BNC117- (Virus 3: resistant, Virus 4: susceptible) and 10-1074- (Virus 3: susceptible, Virus 4: resistant) mediated antibody dependent cellular cytotoxicity (ADCC)(**3B, top**).

Virus 4 also harbours a mismatch to the *env* probe (G13A). IPDA *env* cannot detect Virus 4-infected cells but can detect Virus 3-infected cells (**3B, bottom**). G13A is the most common polymorphism [~5%] in subtype B in the *env* probe region.

Thus, if this person were successfully treated with 10-1074, the IPDA would overestimate the intervention's effect on reservoir size (**3C, left**). But, if the person were successfully treated with 3BNC117, the IPDA would erroneously conclude that the intervention had no effect (**3C, right**).

RESULTS (Figure 4): Secondary primer/probe sets can help address challenge of inter-individual HIV diversity



Secondary primer/probe sets can partially address challenges posed by inter-individual HIV diversity.

Towards this goal, we designed a secondary *env* primer/probe set ~50bp downstream of the IPDA *env* set, but still within the intact-discriminating RRE region.

This secondary *env* primer/probe set was able to rescue *env* detection in 9/9 IPDA *env* false-negative participants (**Figure 4A**, p=0.004).

When applied to 36 individuals whose reservoirs were detectable by IPDA, the secondary *env* primer/probe set failed to detect *env*-positive proviruses in 3 individuals (8%), indicating that this is not a universal solution.

However, in the 33/36 (92%) individuals whose reservoirs were detectable by both assays, secondary *env* measurements did not differ significantly from those of the IPDA *env* (**4B**, p=0.43).

The secondary *env* primer/probe set may therefore be useful in identifying IPDA *env* false-negative results, though, unlike the IPDA, it is unable to discriminate sequences defective due to hypermutation alone.

Additionally, the requirement to discriminate proviral defects in the Ψ region limits the placement and sequence of these primers and probe.

SUMMARY and CONCLUSIONS

Inter-individual HIV diversity poses a challenge to the application of the Intact Proviral DNA Assay (IPDA), where we observed a 28% false-negative rate in our cohort of subtype B-infected individuals from across North America attributable to HIV polymorphism.

Within-host HIV diversity, where IPDA-detectable and undetectable sub-populations co-exist in an individual's reservoir, poses a further challenge to accurate reservoir quantification by IPDA, especially as it is applied to evaluate candidate HIV cure interventions in clinical trials.

Secondary primer/probe sets can be used to mitigate the challenge posed by inter-individual diversity and to identify false-negative results.

Clinical trial participants should be sequenced over the IPDA amplicon regions to ensure accurate interpretation of results.

Given the clear value of the IPDA, collaborative and iterative efforts to refine the assay should be undertaken.