# HIV-1/Mtb Coinfection Selects for bNAb-Resistant Envelopes Revealed by Droplet PCR Sequencing

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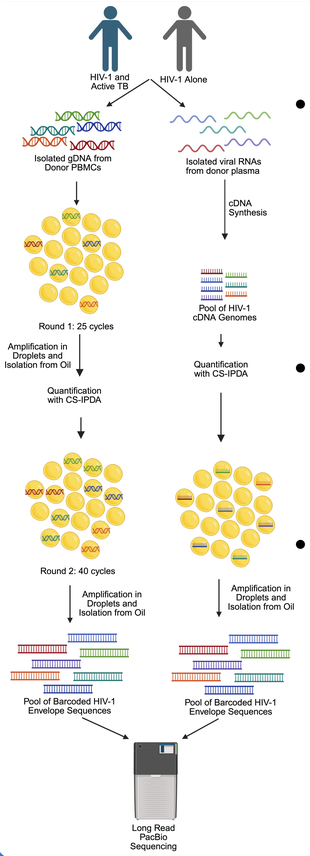
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## Objective

Individuals living with HIV-1 and active tuberculosis (TB) develop broader and more potent neutralizing antibody responses than those living with HIV-1 alone, yet they fail to achieve viral control. We hypothesized that enhanced antibody pressure in HIV-1/Mtb coinfection selects for viral variants with increased resistance to neutralization. To test this, we characterized the HIV-1 quasispecies using a novel droplet digital PCR (ddPCR) approach developed in our laboratory, enabling high-fidelity amplification of thousands of envelope sequences per individual with minimal recombination. The resistance of each sequence was predicted with bNAb-ReP, a machine learning classifier.

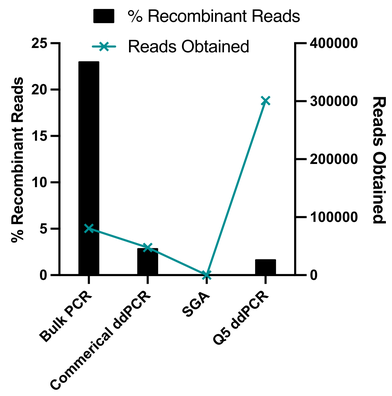
## Methods

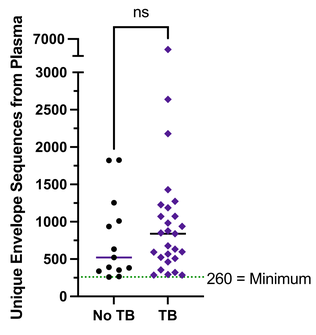


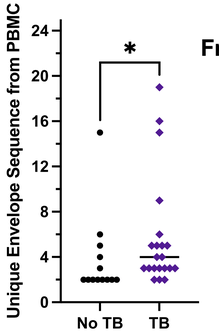

- A maximum input of 3,000 templates/ddPCR was used. We have previously shown that this results in recombination rates ~1%,
- After sequencing, reads were demultiplexed, quality filtered, clustered, frequency filtered
- Following QC, reads were run through bNAb-ReP to predict neutralization sensitivity.

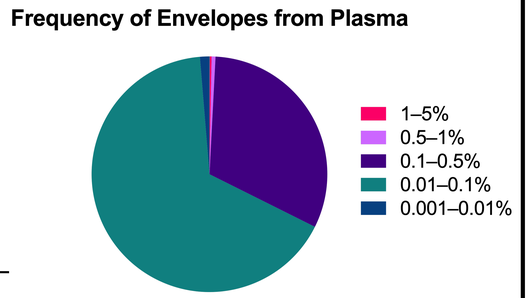
## Results

### Droplet Emulsion PCR Captures Hundreds to Thousands of Rare HIV-1 Envelope Sequences

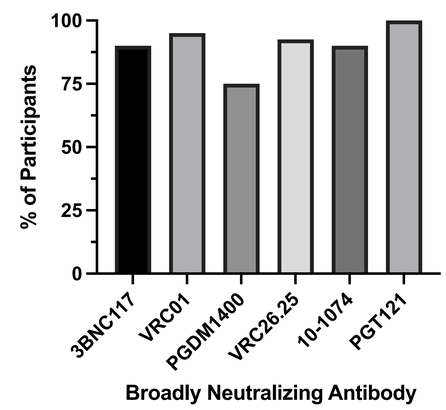


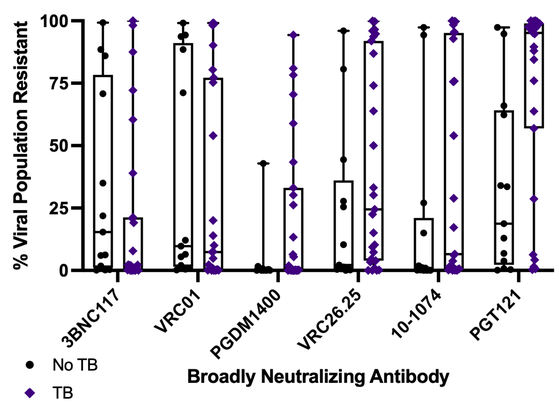



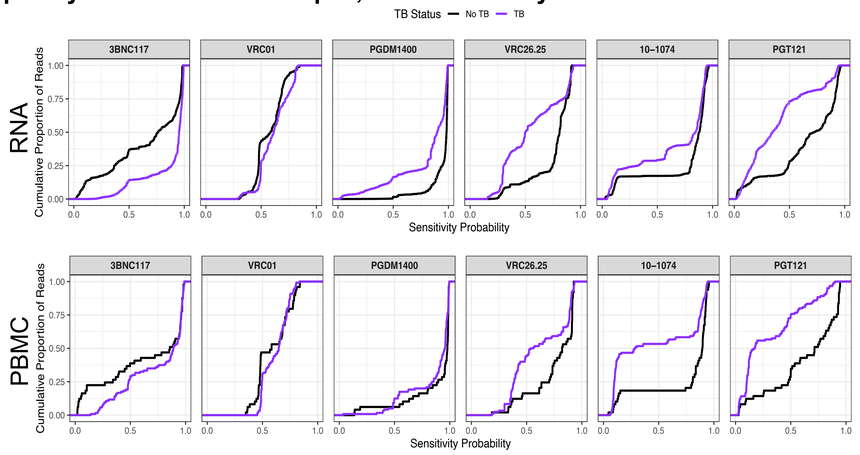




### The Majority of Donors Harbor Resistant Envelopes to bNAbs from Multiple Classes

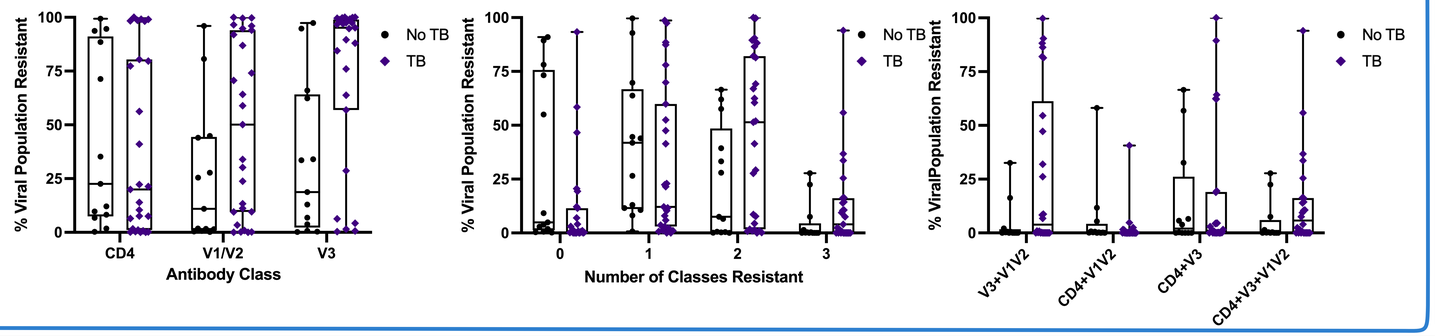



### HIV-1/Mtb Coinfection Changes the Kind and Frequency of Resistant Envelopes, as Predicted by bNAb-ReP




### HIV-1/Mtb Coinfection Selects for Envelope Sequences Resistant to V1/V2 and V3 Classes of Antibodies, as well as Multi-class-resistant Variants, but not CD4bs bNAbs, as Predicted by bNAb-ReP




## Conclusions

- Our novel droplet PCR successfully captures and characterizes hundreds to thousands of unique HIV-1 envelope sequences per individual, the vast majority of which are present at frequencies below 0.5%.
- Most donors, regardless of TB status, harbor resistant variants to every bNAb assayed.
- HIV-1/TB coinfection shapes the circulating HIV-1 quasispecies by selecting for variants which are more likely to be resistant to V1/V2 or V3 targeting bNAbs, but not CD4 targeting bNAbs.
- We observe a higher prevalence of HIV-1 envelopes which are predicted to be resistant to multiple classes of antibodies in HIV-1/Mtb coinfection as compared to HIV-1 alone. Especially V1/V2 + V3 combinations or triple resistant envelopes.

We developed a new method to deeply analyze many variants of HIV-1 circulating within an individual. We found that TB coinfection is associated with HIV-1 variants that are more likely to be resistant to certain bNAbs and combinations of bNAbs, suggesting TB may influence how HIV-1 evolves in the body and responds to selective pressure from neutralizing antibodies.