Q4ddPCR: A Flexible, 4-Target Assay for Scalable High-Resolution HIV Reservoir Profiling

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BACKGROUND

- Precise quantification of intact proviruses is essential for evaluating outcomes in cure directed HIV trials.
- The Intact Proviral DNA Assay (IPDA) excludes most defective proviruses[1] but overestimates intact reservoir size and is prone to failures linked to HIV sequence heterogeneity.
- Mathematical modelling and 4-target Q4PCR predict improved specificity with increased number of targets[2,3]

METHODS

- **Q4ddPCR** uses two sets of 4 primer/probes targeting *env,* packaging signal (*Ψ*), *gag* and *pol,* derived from Q4PCR[3] and IPDA[1] (Fig 1).
- Benchmarked against 3,650 near full-length (NFL) proviral sequences from 13 PWH obtained by Q4PCR that uses the same primers and probes.

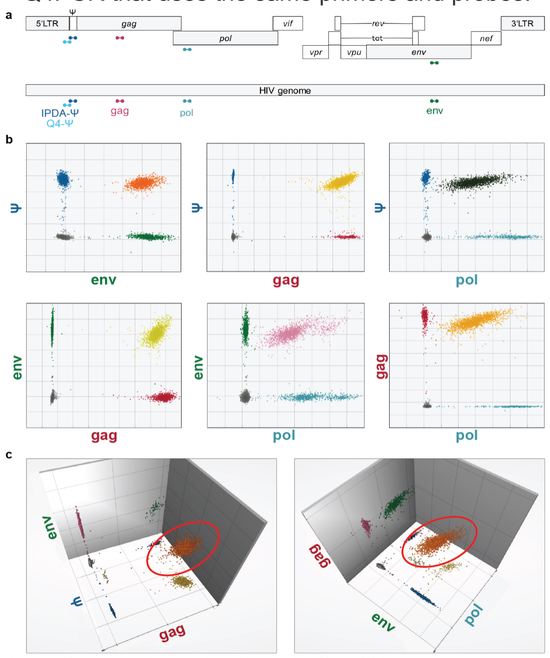


Figure 1 | Development and optimization of the Q4ddPCR

RESULTS

Q4ddPCR validation on sequenced samples from 13 PWH:

- 4-target Q4ddPCR readout highly correlates with sequence-confirmed Q4PCR reservoir estimates (r = 0.72, p < 0.0001)
- Specificity improves with increasing number of targets (42% → 95% intact, Fig. 2a).

Q4ddPCR precisely quantifies intact HIV reservoirs, reduces assay dropout to 5%, tracks reservoir decay and closely correlates with viral outgrowth.

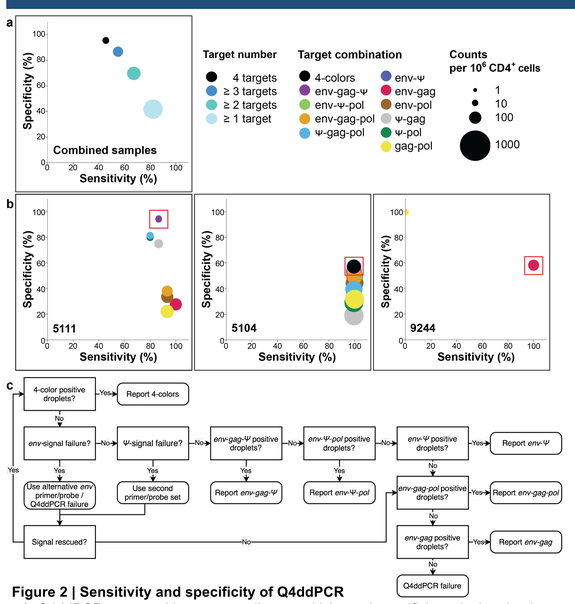


Figure 2 | Sensitivity and specificity of Q4ddPCR
a,b Q4ddPCR counts with corresponding sensitivity and specificity calculated using matched near full-length sequences across 13 PWH. Red squares highlight readout selected by decision tree. **c** Decision tree for reporting intact proviral frequencies.

- Accuracy of Q4ddPCR readouts is participant-specific due to HIV sequence heterogeneity: In cases of *Ψ* dropout, alternative target combinations enable accurate reservoir quantification (Fig. 2b).
- Decision tree as a sequence-informed strategy that maximizes sensitivity and specificity (Fig. 2c).

Q4ddPCR inherits IPDA information and strongly correlates with viral outgrowth assay (QVOA):

- High concordance between Q4ddPCR and IPDA for total HIV DNA and *env-Ψ* readout (Fig. 3a, b) in 27 PWH on ART.
- Higher specificity: 2.5-fold lower intact proviral frequencies than IPDA (range 0.5 - 6.7-fold) (Fig. 3c)
- Q4ddPCR is an estimate for functional reservoir: In 16 PWH, Q4ddPCR correlates with QVOA (r = 0.74, p = 0.001), while IPDA does not (r = 0.34, p = 0.19) (Fig. 3d-f).

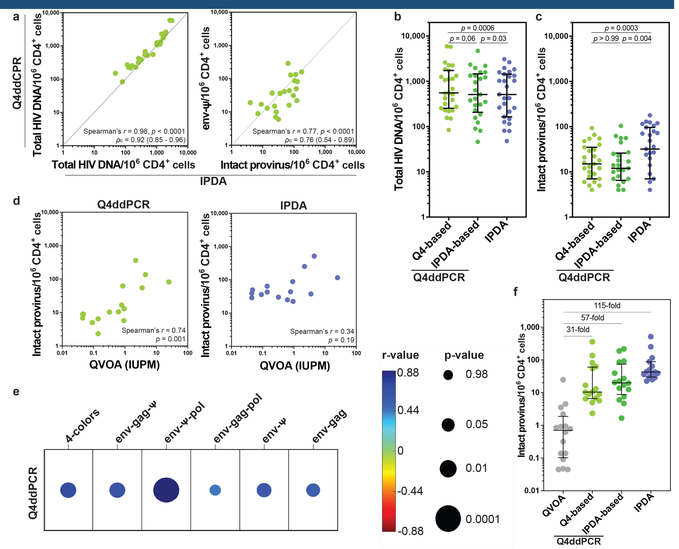


Figure 3 | Q4ddPCR inherits IPDA and correlates with viral outgrowth assay (QVOA)
a,b Q4ddPCR total HIV DNA and *env-Ψ*-readout compared to IPDA in 27 PWH. **c** Comparison of intact proviruses by Q4ddPCR and IPDA. **d-f** Comparison between QVOA, Q4ddPCR and IPDA. **e** Spearman correlation between Q4ddPCR readouts and QVOA. Medians + IQR.

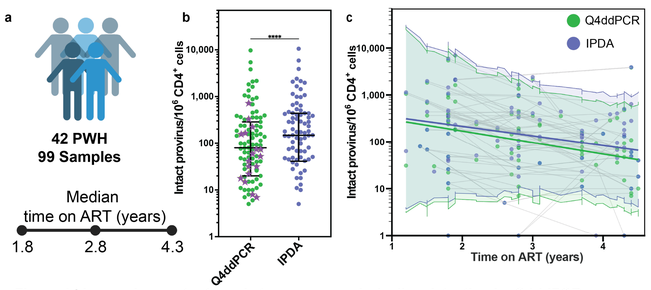


Figure 4 | Lower drop-out rate and steeper reservoir decline detection by Q4ddPCR
a Longitudinal samples from 42 PWH who started ART within ACTG trials. **b** Comparison of intact reservoir measured by Q4ddPCR vs. IPDA. Purple asterisks mark IPDA failures rescued by Q4ddPCR. Medians + IQR. **d** Log-linear mixed-effects model with 95% CI of intact proviral decline by Q4ddPCR and IPDA.

Q4ddPCR reveals lower proportion and steeper decline of intact proviruses compared to IPDA:

- Q4ddPCR rescued reservoir quantification in longitudinal samples of 42 PWH early on ART where IPDA failed: 94% (93/99) vs. 77% (76/99) of samples with quantifiable reservoir (Fig. 4a, b).

- Q4ddPCR revealed steeper reservoir dynamics: 43% annual decline (half-life 1.2 years SE 0.3) vs. 37% by IPDA (half-life 1.5 years, SE 0.5) (Fig. 4c) consistent with mathematical modeling

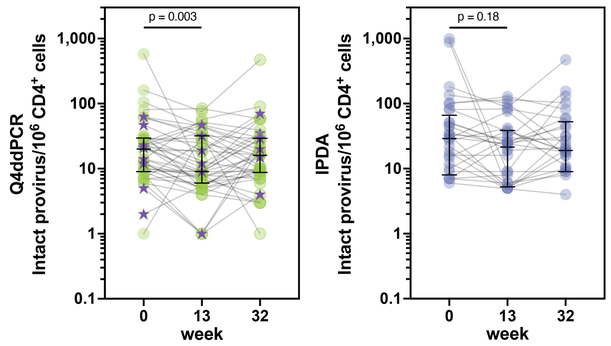


Figure 5 | Q4ddPCR captures reservoir changes in cure directed trial overlooked by IPDA
Q4ddPCR (left) and IPDA (right) intact proviral measurements in ACTG A5386 trial. Purple asterisks mark IPDA failures rescued by Q4ddPCR. Medians + IQR.

Q4ddPCR captures reservoir changes in the ACTG A5386 trial overlooked by IPDA:

- 42 participants received 8 doses of N-803 ± single dose of bNAbs *(see posters **388** and **308**)*
- Q4ddPCR quantified intact proviruses in 96% of samples vs. 72% by IPDA
- Q4ddPCR revealed median reduction of 0.21 $\log_{10}$ (IQR 0-0.38; p = 0.003) in the reservoir after 5 doses of N-803, a change that was not captured by IPDA (Fig. 5)

CONCLUSIONS

- Q4ddPCR is high-throughput and **broadly applicable:** Quantifies intact reservoirs in 95% of samples tested
- **Backward compatible:** Inherits IPDA information (*env-Ψ* readout) while adding sensitivity and specificity.
- Tracks reservoir changes more precisely and **correlates with viral outgrowth**.
- **Confirms predictions from mathematical modeling:** lower proportion of intact proviruses and steeper decay over time compared to IPDA.
- Q4ddPCR **captures reservoir dynamics in interventional trials** that would have been overlooked by IPDA

PLAIN LANGUAGE SUMMARY

- HIV hides in the body even under therapy and this is difficult to measure because HIV looks different across people and even within the same person.
- Q4ddPCR is a new method that can tell more precisely how much "hidden HIV" is in the body and this works for a lot of people with HIV.
- Q4ddPCR helps us researchers to see if new cure strategies are working.

REACH For the Cure
Research Enterprise to Advance a Cure for HIV



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ACTG

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[2] Reeves DB, Gaebler C et al. Nat Commun. 2023, DOI:10.1038/s41467-023-39837-z
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