Development and evaluation of Tasso-M50 method for dried blood viral load detection

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Abstract

Background: At-home blood collection would enable HIV VL assessment for intensive monitoring without frequent access to a clinic—such as for PrEP programs, perinatal or breastfeeding pediatric monitoring, discordant couples, or participation in ART interruption studies. We present the development and qualification of a scalable, high-throughput PCR method using dried blood collected in Tasso-M50 cartridges

Method: We developed a dried blood assay with samples from 21 people with HIV (5% female, 67% African American, mean age 51 years, NCT03588715). At clinic visits, we collected a) capillary blood in two 4-well Tasso-M50 devices and b) matched plasma samples. Between visits, participants self-collected in 2 devices that were mailed back. Automated RNA extraction and duplicate RT-qPCR reads with dual LTR/GAG FAM-labeled primers were performed (up to 16 dried blood PCR reads per timepoint, sourced from ~400 µL blood collection)

Results: Dried blood assay PCR reagents/instruments were qualified on plasma samples (94% sensitivity & specificity, relative to reference lab), and dried blood VL was comparable between home- and clinic-collected samples. In a performance test, the dried blood assay had 99.5% specificity and an estimated 95% limit of detection of 130 c/mL if 16 PCR reads are used. However, 64% of clinic visits with undetected pVL had median M50 reads ≥200 c/mL, due to cell-associated HIV: dried blood VL of these discordant samples correlated with total cellular HIV DNA (r^2 = 0.90) and was stable on suppressive therapy (mean slope –3.0% per week [95% CI –8.3 to +2.5%]). DNase reduced dried blood VL while preserving detectability of RNA standards, suggesting that ~27% of the stable dried blood background in this cohort was contributed by DNA and the remainder by RNA

Conclusions:

- VL can be measured in dried blood collected at home or in the clinic
  - But a large, stable background signal exists, reflecting viral reservoir size
- For dried blood VL assays: Summaries of false positive / negative rate (relative to plasma reference) may be misleading — as can be confounded by variability in reservoir size
- Dried blood can measure total HIV burden (eg, to detect new infections)
  - But detection of viral rebound upon stopping ART would require correcting for each individual's stable background signal

Figure 1. Dried blood assay performance statistics (HIV RNA spiked into whole blood)

		Dried blood assay (Tasso-M50)			Plasma assay (to qualify process used in dried blood assay)
Specificity		99.5% (1/218 blanks read positive Ct = 37.7)			99.5% (1/200 blanks read positive, Ct = 39.4)
Sensitivity			Projected		
	Effective input size	Single PCR read: 15.6 µL	One device (8 PCR reads): 125 µL	Two devices (16 PCR reads): 250 µL	140 µL
	95% LOD: RNA copies per PCR read	19	27	31	34
	95% LOD: c/mL	1200	220	130	250
Precision		Coefficient of variation <40%, in quantifiable range			
Accuracy		Within 80%-125% of nominal, in quantifiable range			

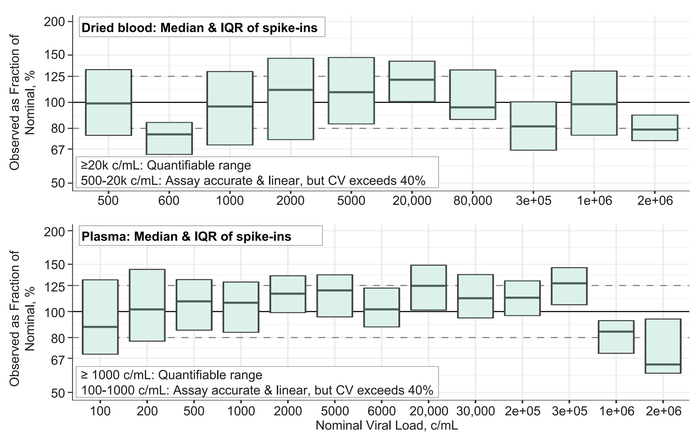
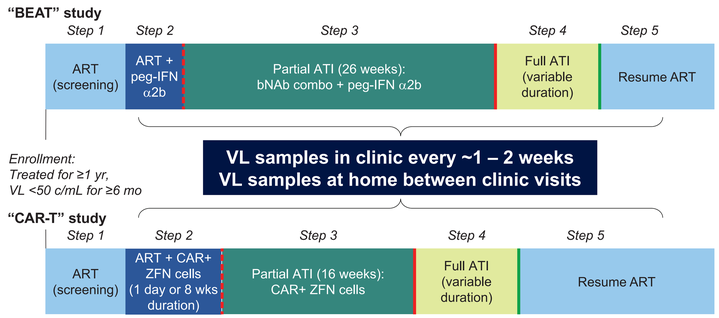
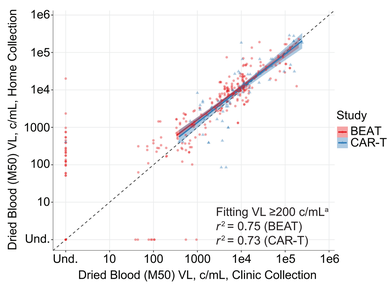


Figure 2. Clinic + home evaluation of Tasso-M50 device



- Fully enclosed capillary blood collection
- 4 polymer tips per device: each absorbs 39 µL blood → extracted twice for 2 PCR reads per tip
- Two devices used per timepoint → up to 16 replicate VL reads
- N=21 participants, mean of 20.5 clinic visits each (every 1 - 2 weeks); devices distributed for home collection between visits
- Total analyzable dried blood VL reads: 5392 (3058 clinic, 2334 home, avg of 6 reads obtained per device)
- Tasso-M50 is a prototype device investigated in this study; Tasso-M20 and Tasso+ devices pictured here

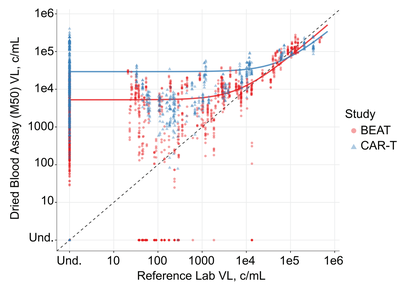
Figure 3. Dried blood VL comparable between home and clinic collections



- Home collection within (80% - 125%) bioequivalence bounds vs clinic collection: 0.85x (95% CI = 0.80, 0.90)[b]
- Only 39/408 (10%) of matched windows[a] are discordant (median readout detected in home or clinic, undetected in the other). Of these:
  - 20 (51%) occur with detected value <200 c/mL
  - 37 (95%) occur with detected value <1000 c/mL

[a]Rolling 2-week windows used to match home and clinic collections for plotting; showing median readout for each window.
[b]Semiparametric model: spline to control for temporal change in VL over time; one effect parameter on home/clinic ratio.

Figure 4. Dried blood VL has large "background signal" compared to matched clinical reference (plasma VL)



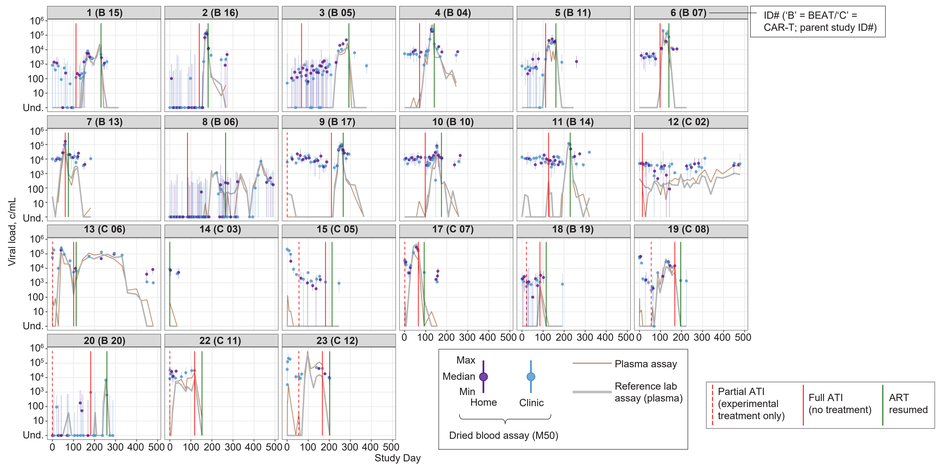
- 64% (121 of 188) of clinic visits with undetected reference lab VL had median dried blood VL ≥200 c/mL
- Typical background signal ~5000 c/mL (BEAT study), ~30,000 c/mL (CAR-T study)[c]

[c]Curves show linear fits on log axes; intercepts provide estimate of typical background signal size in each study. Points show all dried blood assay replicates (up to 16 per timepoint) versus single reference value.

Acknowledgements

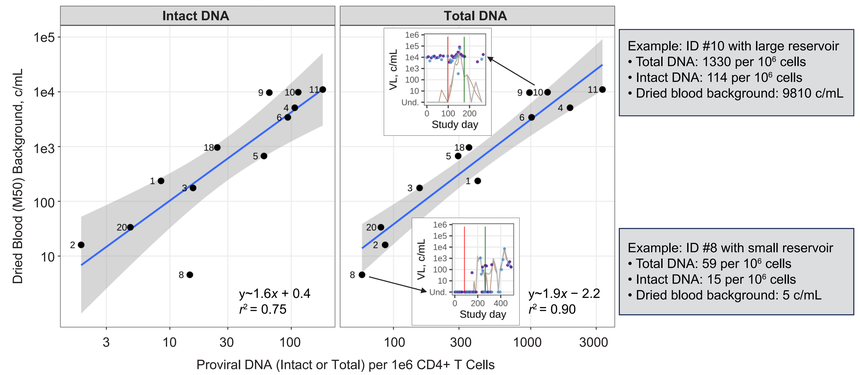
QC of code & dataset: Alejandra Herrera Reyes

Figure 5. VL trajectories for all participants: Background dried blood signal is stable when plasma VL is suppressed



- Background signal shows ~flat slope over time: –3.0% per week [95% CI: –8.3 to +2.5]

Figure 6. Background dried blood signal tracks size of latent reservoir (total or intact cellular DNA)



- Background signal computed as median dried blood VL during suppressive therapy. Where median is undetected, imputation was done on the fraction of replicates positive. Equations shown use $\log_{10}$-scaled values

Figure 7. DNase reduced – but did not eliminate – background dried blood signal

Participant ID	% reduction in dried blood background signal with DNase	% reduction of RNA standards with DNase	Estimated % RNA in this sample
9	70%	42%	53%
18	64%		62%
6	61%		68%
1	59%		71%
7	59%		72%
10	56%		77%
4	53%		82%
11	52%		83%
3	49%		88%
Avg	**58%**		**73%**

- Whole blood samples from 9 participants on suppressive ART: DNase wash reduced dried blood background by 58% (avg.)
  - DNase wash reduced RNA standards by smaller amount (42%)
  - → *Implies signal/background ratio boosted by 38% on average*
- Assuming DNase removes 100% of DNA → typical dried blood background signal in this cohort is ~73% RNA, ~27% DNA

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