Clinical Validation of iMune qPCR as a novel solution to CD4 counting off dried blood spots

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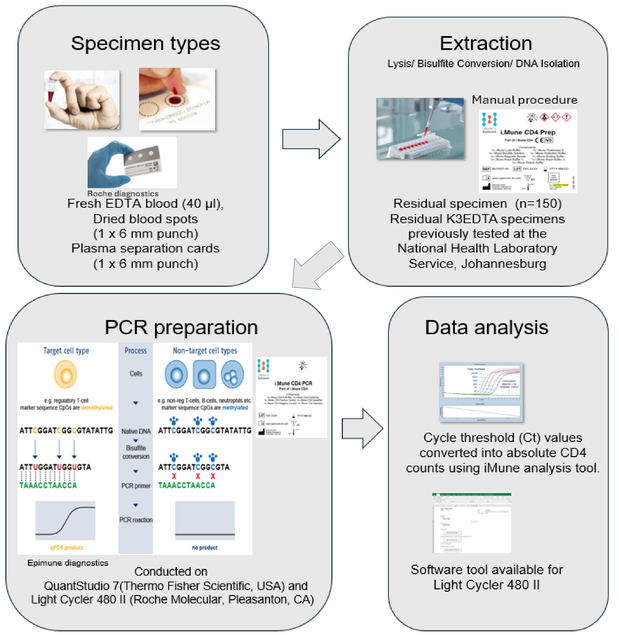
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BACKGROUND

- Quantification of CD4 T helper cells is important for monitoring disease progression and opportunistic infections in people living with HIV (PLHIV)[1].
- However, following the test-and-treat policy implementation by the WHO in 2017[1], there has been a decline in CD4 testing. This led to the discontinuation of two point-of-care (POC) flow Cytometry CD4 tests, the PIMA CD4 (Abbott, IL, USA) and FACSPresto (Becton Dickinson Biosciences, San Jose CA), resulting in an unmet need for remote CD4 services[2], especially in resource-limited settings.
- A novel CD4 enumeration technology, iMune CD4 (Epimune Diagnostics, Berlin, Germany)[3], is designed using epigenetic qPCR which may be performed on fresh and dried blood, making this accessible to testing off dried blood spots and potentially fill testing gaps in global health.
- The study aims to derive a CD4 count off DBS and cobas plasma separation cards (Roche) and compare results to routinely performed flow cytometry. Epigenetic qPCR was also tested on two different qPCR laboratory platforms.

METHODS



Comparator: Pan*Leuco*gated (PLG) CD4 performed on an AQUIOS flow cytometer (Beckman Coulter, Brea, CA)

Figure 1: Study design, qPCR and flow cytometry

qPCR is a viable option for CD4 monitoring off dried blood; eliminating the need for phlebotomy; increasing access to testing; and using existing PCR infrastructure

RESULTS

Analyses presented graphically and in tables as follows:

- Clinical specimens (K_3EDTA) reported a median CD4 count (flow cytometry) of 176 (cells/µl), a range of 5-1361cells/µl and testing performed within 24Hrs.
- qPCR iMune CD4 performed better on DBS than PSC (Figure 2 and Table 1).
- qPCR iMune CD4 manual processing in batches >10 resulted in great variability (Figure 3)
- The overall qPCR error rate was 6% (9/150 samples) for DBS and 16% (24/150) for PSC
- qPCR iMune CD4 was compatible with both the QuantStudio 7 and Light Cycler 480II

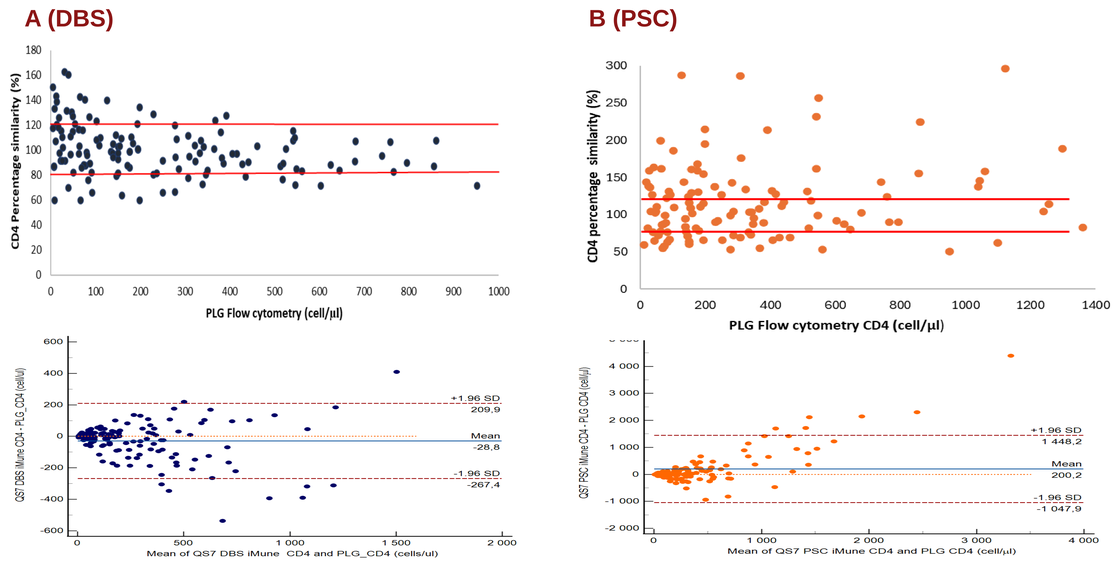


Figure 2: Method Comparison between Flow Cytometry and iMune CD4 Epigenetic qPCR on (A) DBS and (B) PSC (top) percentage similarity plot and (bottom) Bland-Altman plot of agreement.

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CONCLUSIONS

- There is good agreement between DBS iMune CD4 quantification and PLGCD4 Flow Cytometry with a small (but variable) bias of ~28cells/µl and some misclassification generated by higher qPCR results.
- In the absence of POC or remote CD4 testing, the iMune qPCR CD4 offers an alternative solution that is not-reliant on phlebotomy or fresh blood specimens and can be performed on existing laboratory platforms and infrastructure.
- There is greater variance using the PSC ~ 140 cells/µl and differences between the QuantStudio 7 and the LightCycler 480 II that requires automation and optimization.

Table 1: Overall results of the method comparison between the DBS and PSC

parameters	DBS (QuantStudio 7)	PSC (QuantStudio 7)
Concordance correlation (ρc)	0.91 ([CI] 0.88 to 0.93) moderate strength	0.43 ([CI] 0.35 to 0.54) poor strength
Mean bias (cells/µl)	-28.78	200.17
Standard deviation [SD] of the bias (cells/µl)	121.76*	636.76
percentage similarity (%)	102.27	136.30
misclassification CD4<100 cells/µl	56.1% (3/49)	20% (7/35)
misclassification CD4<200 cells/µl	16.48% (15/91)	42.86% (27/63)

* Testing was also performed using a Light Cycler 480 II (Roche) off extracted and stored DNA= 147.16 cells/µl showing increased variability *(possible due to DNA degradation on storage)*

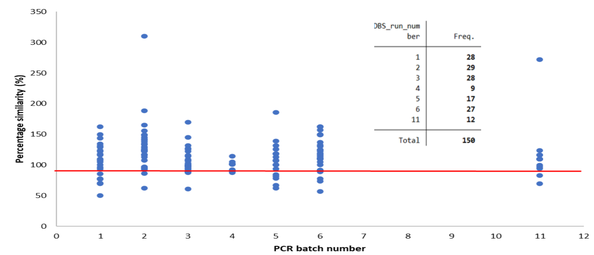


Figure 3: The %similarity of the DBS PCR batches. The key shows the sample size in each batch, with increased variability >10 samples/batch

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2. Syarif O, et al. Resolving the CD4-testing crisis to help end AIDS-related deaths. Lancet Glob Health. 2025.
3. Baron Udo, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. Science Translational Medicine. 2018 Aug.