PE18
Improved assays to measure the inducible latent HIV reservoir

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\textbf{Background:} Precise and practical assays that can reliably measure the impact of a candidate treatment strategy are essential. We improved the standard quantitative viral outgrowth assay (QVOA) and developed a new assay, which promises to be faster, more sensitive, and higher throughput than the standard QVOA.

\textbf{Methods:} Freshly isolated CD4 T cells from 7 ART-suppressed subjects treated during chronic infection were analyzed for total HIV DNA by droplet digital PCR (ddPCR, gag) and our newly developed assays for the inducible HIV reservoir - modified QVOA (mQVOA) and inducible cell-associated RNA expression in dilution (iCARED). For mQVOA, CD4 T-cells in limiting dilution were activated with anti-CD3/CD28 antibodies. After 2 days of culture, MOLT-4/CCR5 cells were added to the culture and cell-free (cf-) RNA was quantified by real-time PCR (Pol) at day 7. Similarly, we used CD3/CD28 co-stimulation for the iCARED assay in the presence of raltegravir. After 3 days of culture, cell-associated (ca-) RNA was quantified by ddPCR (gag and tat-rev). In both cases, we used a magnetic-bead based RNA extraction system (Hologic\textsuperscript{TM}) to specifically extract HIV RNA molecules, making it more sensitive than conventional methods and allowing the testing of large volumes of both cells and culture supernatant.

\textbf{Results:} The median for total HIV DNA was 168 [103-332] copies/106 PBMCs and for mQVOA was 5 [1.7-7.3] infectious units/106 CD4 T cells. There was only a 42-fold difference between the two measures; substantially less than what has been reported previously. In the iCARED assay, the median frequency of cells with inducible ca-RNA was 45 [20-61] cells/106 CD4 T cells, which was 10 times more than the median frequency measured by mQVOA and 4 times less than the median frequency given by total HIV DNA. The latently infected cells detected by iCARED assay was highly correlated with quantification by mQVOA (R=0.89, p=0.007) and HIV DNA (R=0.95, p=0.01).

\textbf{Conclusions:} iCARED is a simple method to quantify the transcriptionally competent latent HIV reservoir. Our results suggest that iCARED, which is more rapid (4 days), less expensive, less cell-demanding and hands on time than QVOA, could prove to be a useful tool for clinical investigations.