

Several candidate HIV vaccines aim to induce virus-specific cellular immunity particularly in the genital tract, typically the initial site of HIV acquisition. However, standardized and sensitive methods for evaluating HIV-specific immune responses at the genital level are lacking. Therefore we evaluated real-time quantitative PCR (qPCR) as a potential platform to measure these responses. β -Actin and GAPDH were identified as the most stable housekeeping reference genes in peripheral blood mononuclear cells (PBMCs) and cervical mononuclear cells (CMCs) respectively and were used for normalizing transcript mRNA expression. HIV-specific cellular T cell immune responses to a pool of optimized CD8+ HIV epitopes (HIV epitope pool) and Staphylococcal enterotoxin B (SEB) superantigen control were assayed in HIV infected PBMC by qPCR, with parallel assessment of cytokine protein production. Peak HIV-specific mRNA expression of IFN γ , IL-2 and TNF α occurred after 3, 5 and 12 hours respectively. PBMCs were titrated to cervical appropriate cell numbers to determine minimum required assay input cell numbers; qPCR retained sensitivity with input of at least 2.5×10^4 PBMCs. This optimized qPCR assay was then used to assess HIV-specific cellular T cell responses in cytobrush-derived cervical T cells from HIV positive individuals. SEB induced IFN γ mRNA transcription was detected in CMCs and correlated positively with IFN γ protein production. However, qPCR was unable to detect HIV-induced cytokine mRNA production in the cervix of HIV-infected women despite robust detection of gene induction in PBMCs. In conclusion, although qPCR can be used to measure *ex vivo* cellular immune responses to HIV in blood, HIV-specific responses in the cervix may fall below the threshold of qPCR detection. Nonetheless, this platform may have a potential role in measuring mitogen-induced immune responses in the genital tract