Table 1: Current viral measurements and strategies to estimate HIV reservoir size**.**

Assay	What it measures?	Advantages	Drawbacks	Involves stimulation and/or expansion?	Available at Virus & Reservoirs Core?
Assays measuring levels of replication-competent virus or intact HIV genomes					
Quantitative Viral Outgrowth assay (QVOA).[1-5]	Frequencies of cells harboring replication-competent HIV that can be induced *ex vivo* to produce infectious virus.	Has been regarded as the definitive assay to measure the size of the replication-competent HIV reservoir; clade independent.	Underestimates the size of the replication-competent HIV reservoir; requires a large number of cells; time-consuming and labor-intensive; cost-intensive.	*ex vivo* stimulation followed by expansion.	*Yes*
Modified QVOA using cell lines supporting HIV replication.[6]	Frequencies of cells harboring replication-competent HIV that can be induced *ex vivo* to produce infectious virus.	Less labor-intensive and more consistent compared to the traditional QVOA; clade independent.	Underestimates the size of replication-competent HIV reservoir; requires a large number of cells; cost-intensive.	*ex vivo* stimulation followed by expansion.	*No*
The qualitative and quantitative viral outgrowth assay (Q2VOA).[7]	Frequency of cells encoding HIV proviral DNA that can be induced *ex vivo*, as well as genetic and potentially phenotypic	Provides additional insights on the qualitative nature of HIV proviral DNA.	Underestimates the size of the replication-competent virus as it misses replication-competent non-induced proviruses, similar to	*ex vivo* stimulation followed by expansion.	*No*

	characterization of latent viruses.		traditional QVOA. Labor, time, and cost-intensive; the sequencing part of the assay could be clade dependent.		
Intact proviral DNA assay (IPDA): HIV DNA by digital droplet PCR targeting multiple regions of proviral DNA to exclude deleted and hypermutated proviruses.[8]	Frequency of cells encoding intact and defective proviral HIV DNA.	Eliminates 97% of defective proviruses and is predicted to overestimate the size of the latent reservoir by only ~ 1.5 fold; simple, medium cost, and fast compared to traditional QVOA.	Sequence polymorphisms could preclude amplification in some patients; alternative primers/probes needed; clade-dependent; does not measure the inducibility of the proviruses.	No stimulation or expansion.	*Yes. Available for HIV+ human cells, SIV/SHIV+ NHP cells*
HIV DNA by limiting dilution four-probe qPCR assay followed by sequence verification of reactions positive for two or more probes (Q4PCR).[9]	Frequency of cells encoding intact and defective proviral HIV DNA confirmed by sequencing.	Estimates levels of replication-competent virus; results are verified by sequencing; sensitive.	Lower throughput and more time-consuming, and labor-intensive compared to IPDA; cost-intensive; clade dependent; does not measure the inducibility of the proviruses; does not provide absolute quantification.	No stimulation or expansion.	*No*

Near full-length individual proviral sequencing (FLIP-Seq).[4,10]	Levels and genetic characteristics of genetically-intact proviruses.	Measures the levels of genetically-intact proviruses in a definitive manner.	Time-consuming and labor-intensive; cost-intensive; requires custom design of primers.	No stimulation or expansion.	*No*
Proviral ultra-deep sequencing.[11]	Could measure levels of genetically-intact proviruses.	Cost-effective; simple compared to single-genome sequencing.	Potential template resampling and PCR errors/bias; cost-intensive; clade dependent.	No stimulation or expansion.	*No*
High throughput integration site sequence analysis.[12] [13]	Distributions of integration HIV proviruses, with linked information on clonal abundance	Efficiently reports locations of integration sites, and associations of insertional mutagenesis and clonal expansion	Requires specialized expertise; cost-intensive; requires workable numbers of proviruses to analyze in samples	No stimulation or expansion.	*Yes*
Matched integration site and proviral sequencing (MIP-Seq). [14]	Individual proviral sequences and corresponding chromosomal integration site.	Investigate chromosomal positioning and integration site features of intact HIV-1 proviruses.	Technically complex and cost-intensive.	No stimulation or expansion.	*No*
Murine viral outgrowth assay (mVOA).[15]	Presence/absence of replication-competent virus within the number of cells/tissues tested.	Can detect low levels of latent infection; clade independent.	Multiple animals are required per sample if a quantitative value is needed; needs a large number of cells; cost-intensive.	*In vivo* expansion.	*No*

Assays measuring inducible translationally- or transcriptionally-competent virus					
Modified QVOA using ultra-sensitive readouts (including QVOA p24 SIMOA and QVOA cfRNA).[16-18]	Frequencies of cells harboring transcription-competent and translation-competent HIV that can be induced *ex vivo*.	Simple, fast, and requires fewer cells compared to QVOA; detects transcriptional-competent and translation-competent virus.	Can detect cells infected with a replication-incompetent virus that is able to produce viral RNA or protein; Semi-labor intensive unless automated; cost-intensive.	*ex vivo* stimulation followed by expansion (unless done in the presence of ART).	*No*
Cell-associated p24 protein quantification. [19]	Productive HIV p24 expression in the presence or absence of *ex-vivo* stimulation.	Sensitive; simple; fast; relatively cost-effective; requires relatively few cells; application across sample types; measurement of productively expressing "active" and "inducible" reservoirs; closer to replication competence when compared to measuring HIV transcripts; biologically relevant target for immune response and immune-based	May overestimate reservoir size as detects translation-competent and replication-competent provirus rather than the replication-competent virus.	Can be done without stimulation but usually done with short *ex vivo* stimulation but no expansion.	*Available at CFAR Immunology Core*

		interventional approaches.			
Induction-based viral RNA reactivation assays (including TILDA).[16,20,21]	The number of cells harboring transcriptionally reactivatable HIV tat/rev msRNA.	Simpler, faster, and requires fewer cells compared to QVOA; can be useful in measuring the response to LRAs *ex vivo*.	Measure inducible levels of HIV transcripts (transcriptional competence) without distinction of defective or replication-competent (replication competence); cost-intensive; semi-labor intensive unless automated.	short *ex-vivo* stimulation but no expansion.	*No*
Single-cell analysis of inducible virus (including FISH/flow). [22]	Measures the frequency of cells undergoing HIV transcription and/or translation upon stimulation at the single-cell level.	Single-cell insights of HIV transcription and/or translation and phenotypic characterization of individual cells; clade independent; relatively cost-effective.	Do not measure replication competence.	Can be done without stimulation but usually done with short *ex-vivo* stimulation but no expansion.	*No*
Assays measuring constitutive levels of HIV DNA and cell-associated HIV RNA					
Cell-associated HIV RNA by real-time or droplet digital PCR.[23-30]	Levels of HIV transcripts (a surrogate of transcription-competent cellular HIV) without distinction of	Fast, relatively cost-effective, and sensitive; cell-sparing; application across sample types; Different HIV transcripts	Overestimates the size of the latent HIV reservoir. Doesn't measure translation- or replication-	No stimulation or expansion.	*Yes. Available for HIV+ human cells, SIV/SHIV+ NHP cells*

	defective, translation-competent, or replication-competent.	can be measured to indicate the degree of HIV transcriptional activity; may provide a surrogate measure for reservoir size as levels of HIV RNA during ART predict time to viral rebound upon treatment cessation.	competence; clade dependent.		
HIV DNA by real-time PCR or droplet digital PCR.[23,24,26,31-37]	Levels of selected regions of total, integrated, or circular HIV DNA measure without distinction of defective or replication-competent provirus.	Fast, cost-effective, and sensitive; cell-sparing, application across sample types; most available assays span across different clades.	Vastly overestimates the size of the HIV reservoir, as only a small fraction of HIV genomes is able to be reactivated upon *ex vivo* stimulation to produce replication-competent virus.	No stimulation or expansion.	*Yes. Available for HIV+ human cells, SIV/SHIV+ NHP cells*
***In situ* hybridization-based assays, e.g., DNAscope and RNAscope.**[26,38,39]	Cellular location and levels of HIV sequences associated with integration (DNA) or transcription (RNA) without distinction of defective or replication-competent.	Visualize and phenotypically characterize infected cells in tissues; allow for a better understanding of the anatomical distribution of infected cells *in vivo*; cost-	Do not measure replication competence; clade dependent.	No stimulation or expansion.	*No*

		effective; near sensitivity to qRT-PCR.			
Assays measure the *in vivo* burden of HIV reservoirs					
Ultra-sensitive residual viremia.[40-42]	The release of HIV particles *in vivo* during ART, without distinction of tissue origin.	Could represent virus release from stable reservoirs, including these in tissues; relatively cost-effective.	Requires large volumes of plasma or body fluid; limited dynamic range; the relationship between the HIV reservoir and low-level viremia during ART viremia is unclear; clade dependent.	No stimulation or expansion.	*No*
Common strategy to test for viral burden on ART yet not an "Assay," included here for comparison only: **Analytical Treatment Interruptions (ATI).**[43,44]	Systemic virus replication *in vivo* after ART-cessation, without distinction of tissue origin.	Determines the duration of HIV remission upon the cessation of ART. It is the most clinically relevant measure of the impact of interventions on the total body burden of HIV infection.	Potential clinical risks to individuals stopping therapy and to their partners; clinically demanding; substantial reservoir reductions are needed to produce significant delays in viral rebound, so unlikely any current interventions could result in a significant delay in viral rebound. Effort and monitoring cost-intensive	*In vivo* expansion.	-

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