

Table of Assays: Current HVTN LC assays, sample types and validation status.

Assay Category	Assay Description	B	MS	MB	LN	
Antibody	Ab binding to Env and specific epitopes	IgG and IgA isotype by Binding Ab Multiplex Assay (BAMA)	V	Q	S	
		Ig subclass by Binding Ab Multiplex Assay (BAMA)	Q	Q		
		Peptide microarray (mapping of linear epitopes)	Q	Q		
		bnAb precursor binding/stabilized Env trimer	Q			
	Ab binding to viral Env	Infected Cell Ab Binding Assays (ICABA)	S	S		
		Virus capture (infectious virus particle)	S	S		
	Ab binding avidity	Avidity index for IgG1-4, IgA1-2 by BAMA	Q			
		Polyclonal on-rate (Ka) and off-rate (Kd), dissociation phase area under the curve (AUC) by BLI	Q			
	nAb and HIV-1 inhibition	Tier 2 neutralization by TZM-bl	V			
		Expanded neutralization panel for breadth, epitope mapping, precursor detection	Q			
		Ab-mediated inhibition of ex vivo mucosal HIV infection in human explants			S	
	Fc-binding interactions	FcR engagement: FcγR1, FcγRIIa,b, FcγRIIIa, FcRα, FcRn (binding by BAMA; avidity by BLI)	S	S		
		Complement (C') binding	S	S		
		Mucin binding and glycan profiling	S	S		
	ADCC	Infected cell assay (Luc) with NK cells	Q	Q		
		gp120-coated cell assay (GTL) with NK cells	Q	Q		
	Phagocytosis	Monocyte (ADCP) or Neutrophil (ADNP) phagocytosis of Env, virion, or infected cells	Q			
	Infused mAb	mAb PK levels <i>in vivo</i>	V	S	S*	
Anti-drug Ab detection		Q	S			
mAb distribution/localization by IHC				S		
Cellular	Antigen-specific by flow cytometry	T-cell frequency based on IL-2/IFN-γ expression by intracellular cytokine staining (ICS)	V		S	
		T-cell polyfunctionality and phenotype including memory, Tfh, Treg, exhaustion, NKT & γδ T cells	Q		S	
		Antigen- and epitope-specific B-cell phenotyping by flow cytometry	Q		S	S
		Activation induced marker (AIM) assay	Q			S
		Epitope-specific T-cell characterization and MHC restriction analysis by ICS	Q			
	Antigen-specific by ELISpot	T-cell frequency based on IFN-γ expression by ELISpot	V			
		T-cell specificity by epitope mapping	Q			
		B-cell frequency by ELISpot	V			
	Soluble proteins	Interferon Gamma Release Assay (IGRA): QuantiFERON for TB sensitization	Q			
		Antigen-specific cytokine expression: Multiplex Protein Detection Assay (MPDA) on MSD platform	Q			
		Concentration of soluble factors (e.g. cytokines, antimicrobials, hemoglobin) by MSD or ELISA	Q	S		
	Phenotyping by flow cytometry	Cell population frequencies and concentrations by Trucount	Q			
		T, B, NK, DC, myeloid and other cells and markers of interest (e.g., innate, maturation, activation)	S		S	S
	Functional	CD8+ T-cell mediated HIV suppression (VIA), cytotoxicity and avidity	S			
		Proliferative capacity of T cells using CFSE by flow cytometry	Q			
		Innate effector cell function (cytokine, degranulation, NK cell cytotoxicity)	S			
	DNA/RNA	Transcriptional profiling of bulk populations or single cells	S		S	S
		T and B cell receptor sequence analysis	S		S	S
IHC/Histology	Leukocytes, HIV-infected cells, inflammation			S	S	
Other	Host genetics	S				

Sample types: **Blood cells/plasma/serum (B)** **Mucosal secretions (MS)** **Mucosal biopsy/lysate (MB)** **Lymph node aspirates (LN)**

Validation status: **V**=validated, **Q**=qualified, **S**=standardized

* standardization in progress for biopsy lysates