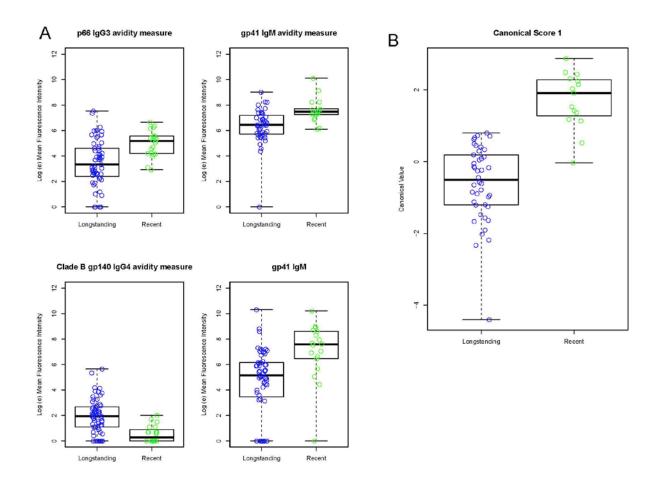
Several cohorts contributed specimens to this study through contributions to the CEPHIA specimen repository: UCSF Options and Scope Studies (San Francisco, US); AMPLIAR study group (Porto Alegre and Sao Paulo, Brazil); IAVI protocol C, ¿Sabes? (IMPACTA, Lima, Peru); CAPRISA (KwaZulu Natal, South Africa); GAMA (Manhica, Mozambigue).

The Consortium for the Development and Performance of HIV Incidence Assays (CEPHIA) is comprised of the authors and: Tom Quinn and Oliver Laeyendecker from John Hopkins University; David Burns from the National Institutes of Health; Jesus Maria Garcia Calleja from the World Health Organization; Tim Hallett from Imperial College London; Sherry M. (Michele) Owen. Bharat Parekh and Connie Sexton from the Centers for Disease Control and Prevention: Anatoli Kamali from the International AIDS Vaccine Initiative; David Matten and Hilmarie Brand from the South African Centre for Epidemiological Modelling and Analysis; Gary Murphy and Megha Mittal from Public Health England; Mila Lebedeva and Dylan Hampton from the Blood Systems Research Institute. Contributors from the University of California, San Francisco included Frederick M. Hecht, Lisa Loeb and Lisa Harms from the Options Study and Steven G. Deeks, Jeffrey N. Martin and Rebecca Hoh from the SCOPE Study University of California, San Francisco. Contributors from the AMPLIAR study group included Esper G. Kallas, Zelinda Bartolomei and Natalia Cerqueira from The University of Sao Paulo, Kellin Zabtoski and Rita de Cassia Alves Lira from Grupo Hospital Conceição, and Rosa Dea Sperhacke, Leonardo R. Motta, and Machline Paganella from the Universidade Caxias Do Sul. Contributors from CAPRISA included Salim S. Abdool Karim, Quarraisha Abdool Karim, Thumbi Ndungu, Nelisile Majola and Natasha Samsunder from the University of Kwazulu-Natal. Contributors from the GAMA Study included Inácio Mandomand and Eusebio V. Macete from Fundacao Manhica. Contributors from the Sabes cohort included Jorge Sanchez and Javier Lama from Asociación Civil Impacta Salud y Educación (IMPACTA). Maria R. Capobianchi contributed from the National Institute for Infectious Diseases "L. Spallanzani" Rome; and Barbara Suligoi contributed from Istituto Superiore di Sanità, Rome.



C

		Assigne		
		Longstanding	Recent	Total
dno	Longstanding	42	0	42
Gro	Recent	2	13	15
,	Total	44	13	57

Total misclassification

3.5% (2/57)

Supplemental Figure 1. Multiple sets of 4 HIV-1 specific analytes classify recent infection with 0% False Recent Rate

An array of 282 antigen-antibody combinations was tested via Binding Antibody
Multiplex Assay (BAMA) and subsequently analyzed using discriminant function
analysis. **A.** The top measurements resulting in an False Recent Rate (FRR) of 0%, **B.**Canonical 1 Score for one of the top solutions includes p66 IgG3 avidity, subtype B
Transmitted Founder (T/F) WITO gp140 IgG4 avidity, gp41 IgM binding, and gp41 IgM
avidity. **C.** Group = Classification based on days from Estimated Date of
Seroconverstion (EDSC) less than or greater than 270 days. Assigned group =
classification predicted by canonical values obtained via BAMA.

	gp140	gp120	C1/V1	V2
Subtype A/AE	9004S,	A244, 254008, 51802	A244	A244, C2101, 06RUSPR163lorll3V2, 191084, 254006P00Ra, 9004SS, C3347
Subtype B	WITO4160, SC42261 RHPA4259	6240, 63251, MN, TT31, BORI	MN	WITO4160, BCaseA_V1_V2, 700010058, REJO4541
Subtype C	1086, CH505 BF1266	1086, 96ZM651, TV1c8		96ZM9651, 1086, BF1266, Ce704010042, 001428
CRF07 B/C		CNE20 BJOX002000		BJOX002000
Consensus	A1 Con, AE Con, B Con, C Con, ConS	Con6	BC AE	AE, B, C

Supplemental Table S1. HIV-1 envelope sequence names and subtypes for down-selected envelope proteins (i.e. gp140, gp120) and epitopes (C1/V1 and V2) regions. In addition to multiple subtypes (A, CRF01-AE, B, C, CRF07-BC) and both genders (M, F), envelope sequences represent infections from multiple transmission routes (i.e. heterosexual transmission, homosexual transmission, breastfeeding, and IV drug use), multiple regions (China, India, Kenya, Malawi, Russian Federation, South Africa, Thailand, Trinidad and Tobago, Uganda, United States, Zambia) and Fiebig Stage of infection (i.e. I, I/II, II, III, IV, V, and VI)

	gp140	gp120	C1/V1	V2	V3	C5	gp41*
Consensus	A1 Con,	Con6	ВС	AE, B, C	A, B, C,	AE,	AE, B,
	AE Con, B		ΑE		CRF01AE,	В,	С
	Con, C				D, M	С	
	Con,						
	ConS						

Supplemental Table S2. Consensus sequence names for down-selected proteins (*i.e.* gp140, gp120) and C1/V1, V2, V3, C5 and gp41 peptide regions. CD4BS and CD4i antigens also included (HxB2new8bcore1420R, RSC3, RSC3delta371, RSC3delta371_P363N, provided by Dr. John Mascola, Vaccine Research Center). * Sequences within the gp41 region also included the following:

KQLQARVLAVERYLKDQQLLG; LQARVLAVERYLKDQKFLGLWGCSGKIICTTAAP; KQLQARVLAVERYLKDQQLLGIWGCSGKLICTTAV;

LQARILAVERYLKDQQLLGIWGCSGKLICTTTAP; and KQLQARVLAIERYLKDQQLLG)

Protein	Supplier
p31	Jena Biosciences
p66 (RT)	Protein Sciences
p24 (Gag)	BD Biosciences

Supplemental Table S3. Non-Envelope sequences for down-selected antigen panel.