A Recombinant HIV-1 Gag Virus Panel for the Evaluation of p24-Antibodies and HIV Diagnostic Tests

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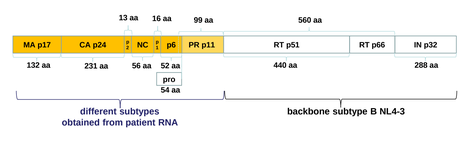
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Background

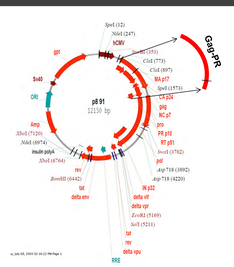
Diagnostically, the HIV-1 p24 protein is currently only used in HIV-1/2 Ab/Ag combination tests (4th generation) or single antigen tests at the time of adult diagnosis. However, there is growing interest in new test development to extend its use to diagnosing paediatric HIV infection and disease monitoring as inexpensive alternatives to PCR-based tests. Regarding the global HIV-1 diversity, such tests should detect all subtypes with similar sensitivity. Readily available, inexhaustible tools for subtype evaluation and test comparisons are a prerequisite for efficient test development. To this end, we developed a panel of 44 recombinantly expressed virus-like-particles (VLPs), which contain the Gag-Protease region of HIV-1 subtypes A, B, C, D, F, G, H, circulating recombinant forms 01_AE, 02_AG, BF/BG, and group O. We characterised 10 mono- or polyclonal antibodies (mAb, pAb) for their subtype-binding profiles and initiated an evaluation of commercially available 4th generation tests and single antigen tests.

Panel Generation

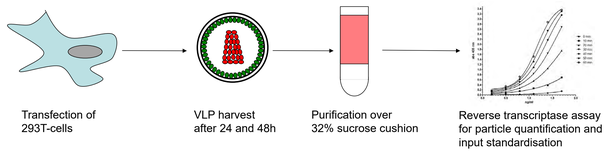
Cloning strategy Gag-PR



p160

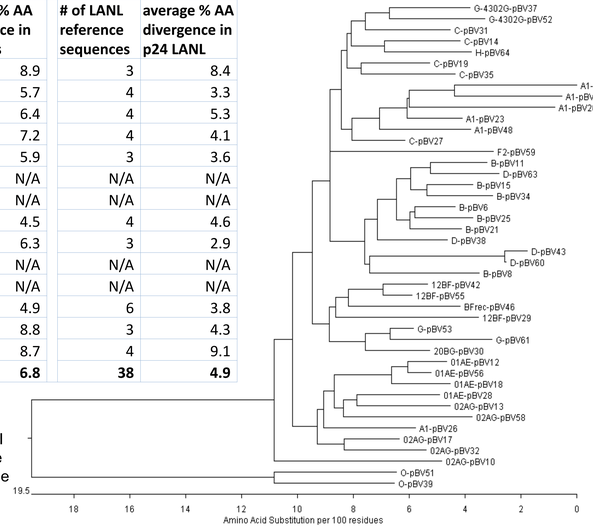


Transfection, purification, quantification



HIV-Gag Panel Members

	# of VLPs	average % AA divergence in p24 VLPs	# of LANL reference sequences	average % AA divergence in p24 LANL
A1	6	8.9	3	8.4
B	7	5.7	4	3.3
C	5	6.4	4	5.3
D	4	7.2	4	4.1
CRF12_BF	3	5.9	3	3.6
BFrec	1	N/A	N/A	N/A
F2	1	N/A	N/A	N/A
G	2	4.5	4	4.6
G/CRF43_02G	2	6.3	3	2.9
CRF20_BG	1	N/A	N/A	N/A
H	1	N/A	N/A	N/A
CRF01_AE	4	4.9	6	3.8
CRF02_AG	5	8.8	3	4.3
O	2	8.7	4	9.1
Grand Total	**44**	**6.8**	**38**	**4.9**



Subtyping: NCBI genotyping tool
Alignment: Jotun Hein sequence alignment in DNASTAR Lasergene MegAlign

AA: amino acid
LANL: Los Alamos National Library

Subtype-Binding Profiles of Anti-p24 Antibodies

name	species	isotype	concentration	company	dilutions tested
BDI690	mouse	IgG_1	100 ug/ml	Santa Cruz sc-57827	5, 1, 0.2 ug/ml
YDHIVgp24	mouse	IgG_1	100 ug/ml	Santa Cruz sc-73300	5, 1, 0.2 ug/ml
N29	mouse	IgG_1	1 mg/ml	Abcam ab63959	5, 1, 0.2 ug/ml
BC1071	mouse	IgG_1	1 mg/ml	Aalto	5, 1, 0.2 ug/ml
LH104-E	mouse	IgG_1	supernatant	NIBSC ARP319	1:10, 1:50, 1:250
AG3.0	mouse	IgG_1	supernatant	NIH 4121	1:10, 1:50, 1:250
3D3	mouse	IgG_{2b}	ascitic fluid	NIBSC ARP314	1:10, 1:50, 1:250
S142	sheep	polyclonal	supernatant	NIBSC ARP406	1:200, 1:1000, 1:5000
D7320	sheep	polyclonal	1 mg/ml	Aalto	5, 1, 0.2 ug/ml
Pooled plasma	human	polyclonal	plasma	HIV-1+ individuals	1:100, 1:500, 1:2500

Method VLP-coated ELISA

Coating: Input-standardised quantities of VLPs (10 ng/ml RT) were lysed and heat-denatured prior to coating on 384-well plates over night. **Antibody binding:** Three dilutions per primary antibody were tested and detected with an anti-mouse-HRP-coupled secondary antibody. **Evaluation:** S/Co ratios were calculated using absorbance ratios of coated to non-coated wells for matched antibody dilutions. S/Co ratios ≥ 2 were considered positive and scored as 1. Scores for all three dilutions were added. If none of the three antibody dilutions achieved a S/Co ratio ≥ 2, the total score was 0. **Confirmation:** VLPs scoring 0 were additionally tested by Western Blot, and those which were undetectable by both methods are marked with a star in the table.
Unique amino acid difference in p24 within the respective antibody epitopes (or peptide sequences used for immunisation), are listed in the columns to the right.

Scores of antibody-binding in VLP-coated ELISA

	BDI690	YDHIV-1 gp24	N29	BC1071	LH104-E** *	AG3.0	3D3 ***	S142	D7320	pooled plasma	% sensitivities mAb	% sensitivities pAb
A1-pBV20	2	2	3	3	*0	3	0	3	2	2	71.4	100.0
A1-pBV22	2	0	2	3	*0	2	0	2	2	2	57.1	100.0
A1-pBV23	2	2	3	3	0	3	0	3	3	2	71.4	100.0
A1-pBV26	0	2	3	3	0	2	0	3	3	3	57.1	100.0
A1-pBV33	*0	2	3	*0	0	1	0	2	2	2	42.9	100.0
A1-pBV48	1	2	3	3	0	0	0	3	3	2	57.1	100.0
								average sensitivity subtype A1			59.5	100.0
B-pBV6	2	3	3	3	1	0	3	3	2	2	85.7	100.0
B-pBV8	2	2	3	3	1	*0	2	3	3	3	85.7	100.0
B-pBV11	3	3	2	3	2	3	3	3	3	3	100.0	100.0
B-pBV15	1	2	3	3	1	1	3	3	3	2	100.0	100.0
B-pBV21	0	2	3	3	1	0	2	3	3	1	71.4	100.0
B-pBV25	0	2	3	3	0	0	2	3	2	2	57.1	100.0
B-pBV34	1	2	3	3	2	1	3	3	3	2	100.0	100.0
								average sensitivity subtype B			85.7	100.0
C-pBV14	1	2	3	3	0	0	2	3	2	1	71.4	100.0
C-pBV27	2	2	2	3	0	2	1	3	3	2	85.7	100.0
C-pBV31	2	2	3	3	*0	2	2	3	3	3	85.7	100.0
C-pBV35	2	2	3	3	*0	2	0	3	2	2	71.4	100.0
C-pBV19	2	2	3	3	0	2	0	3	2	2	71.4	100.0
								average sensitivity subtype C			77.1	100.0
D-pBV38	1	2	3	3	1	2	0	3	3	3	85.7	100.0
D-pBV63	0	1	3	3	0	0	1	3	3	0	57.1	66.7
D-pBV43	3	*0	3	3	2	3	0	3	3	3	71.4	100.0
D-pBV60	0	*0	3	3	0	0	0	3	3	0	28.6	66.7
								average sensitivity subtype D			60.7	83.3
BFrec-pBV46	3	3	3	3	1	3	3	3	3	3	100.0	100.0
								sensitivity subtype BFrec			100.0	100.0
12BF-pBV55	2	0	3	3	*0	3	3	3	3	3	71.4	100.0
12BF-pBV29	2	2	3	3	1	3	0	3	2	2	85.7	100.0
12BF-pBV42	3	*0	3	3	1	3	3	3	3	3	85.7	100.0
								average sensitivity subtype CRF12_BF			81.0	100.0
F2-pBV59	2	2	3	3	1	*0	3	3	3	2	85.7	100.0
								sensitivity subtype F2			85.7	100.0
G-pBV53	2	2	2	3	0	1	0	3	2	2	71.4	100.0
G-pBV61	1	1	1	3	*0	0	*0	3	*0	3	57.1	66.7
								average sensitivity subtype G			64.3	83.3
G/43_02G-pBV37	2	2	2	3	0	2	2	3	3	2	85.7	100.0
G/43_02G-pBV52	0	0	2	3	*0	0	0	2	1	1	28.6	100.0
								average sensitivity subtype G/CRF43_02G			57.1	100.0
20BG-pBV30	2	2	3	3	0	2	3	3	3	2	85.7	100.0
								sensitivity subtype CRF20_BG			85.7	100.0
H-pBV64	2	2	3	3	0	1	0	3	3	2	71.4	100.0
								sensitivity subtype H			71.4	100.0
01_AE-pBV28	2	2	2	3	0	2	1	2	2	1	85.7	100.0
01_AE-pBV56	2	2	3	3	1	3	0	3	3	3	85.7	100.0
01_AE-pBV12	2	2	2	3	1	2	0	2	3	2	85.7	100.0
01_AE-pBV18	3	2	2	3	1	3	0	3	2	3	85.7	100.0
								average sensitivity subtype CRF01_AE			85.7	100.0
02_AG-pBV10	0	0	0	3	0	0	0	2	*0	1	14.3	66.7
02_AG-pBV13	0	2	3	3	1	0	0	2	2	2	57.1	100.0
02_AG-pBV17	0	0	*0	3	0	0	0	1	0	1	14.3	66.7
02_AG-pBV32	0	1	1	3	0	0	0	2	2	1	42.9	100.0
02_AG-pBV58	2	1	*0	3	0	2	*0	2	2	3	57.1	100.0
								average sensitivity subtype CRF02_AG			37.1	86.7
O-pBV39	3	3	1	3	3	3	*0	3	2	3	85.7	100.0
O-pBV51	3	3	0	3	2	3	*0	3	1	3	71.4	100.0
								average sensitivity group O			78.6	100.0
ELISA:												
# of VLPs detected	33	36	40	43	19	29	19	44	41	42		
% VLPs detected	75.0	81.8	90.9	97.7	43.2	65.9	43.2	100.0	93.2	95.5		
ELISA + Western Blot:												
# of VLPs detected	43	40	41	43	37**	42	40**	44	42	44		
% VLPs detected	97.7	90.9	93.2	97.7	84.1	95.5	90.9	100.0	95.5	100.0		

Unique p24 AA differences in epitopes

YDHIV-1 gp24	BC1071	AG3.0	3D3	D7320
	D81V			