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

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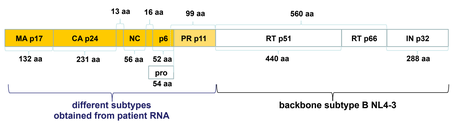
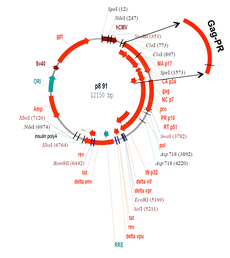
Background

The HIV-1 p24 antigen is used during HIV diagnosis in 4th generation antigen/antibody combination tests and antigen-only tests. High sensitivity and subtype breath is an important feature for early detection of infection and the potential extension of the antigens' use to disease monitoring. To easily evaluate diagnostic tests as well as existing and newly developed anti-p24 antibodies for their Gag sensitivity and subtype breath, we developed a panel of 43 recombinantly expressed virus-like particles (VLPs) expressing the Gag-PR part of subtypes A-H, CRF01_AE, CRF02_AG,CRF12_BF, CRF20_BG and group O.

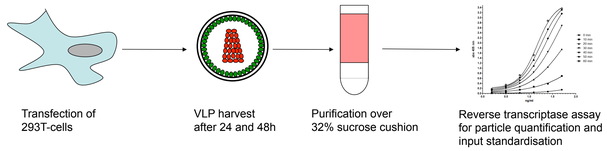
Panel Generation

Cloning strategy Gag-PR





Transfection, purification, quantification

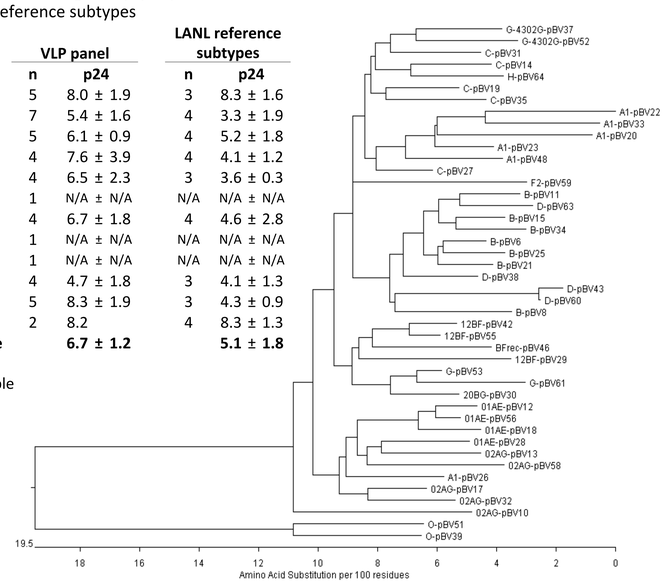


HIV-Gag Panel Members

Amino acid sequence divergence (%) of p24 in VLP panel and LANL reference subtypes

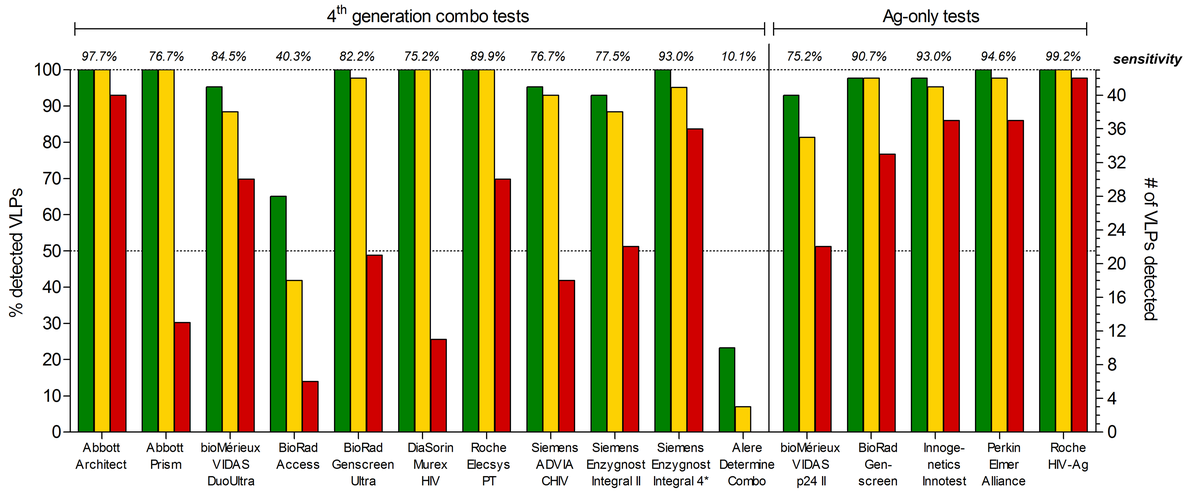
	VLP panel		LANL reference subtypes	
	n	p24	n	p24
A1	5	8.0 ± 1.9	3	8.3 ± 1.6
B	7	5.4 ± 1.6	4	3.3 ± 1.9
C	5	6.1 ± 0.9	4	5.2 ± 1.8
D	4	7.6 ± 3.9	4	4.1 ± 1.2
CRF12_BF	4	6.5 ± 2.3	3	3.6 ± 0.3
F2	1	N/A ± N/A	N/A	N/A ± N/A
G	4	6.7 ± 1.8	4	4.6 ± 2.8
CRF20_BG	1	N/A ± N/A	N/A	N/A ± N/A
H	1	N/A ± N/A	N/A	N/A ± N/A
CRF01_AE	4	4.7 ± 1.8	3	4.1 ± 1.3
CRF02_AG	5	8.3 ± 1.9	3	4.3 ± 0.9
group O	2	8.2	4	8.3 ± 1.3
weighted average		**6.7 ± 1.2**		**5.1 ± 1.8**

N/A = not applicable



Subtyping: NCBI genotyping tool
Alignment: Clustal Omega alignment MegPro
LANL: Los Alamos National Laboratory

Diagnostic HIV Tests



50 IU/ml
10 IU/ml
2 IU/ml

Input conversion

WHO IU/ml	p24 pg/ml	RT ng/ml
50	257.5	0.025
10	51.5	0.005
2	10.3	0.001

VLP input standardisation

The WHO p24 international standard (NIBSC 90/636) was quantified in parallel with four subtype B VLPs (6, 8, 11, 15) to calculate the relationship between RT-activity and p24 content of the WHO standard and the VLPs. The concentration of 2 IU/ml WHO p24 standard (minimal test-sensitivity required for CE-marking) was equivalent to the activity of 0.001 ng/ml recombinant HIV RT (Roche) and 10.3 pg/ml p24 (see table to the left). For diagnostic test evaluation, three concentrations for each VLP were prepared in negative human plasma.

Total numbers of VLPs detected per input concentration and test

	4th generation combo tests											antigen-only tests						
	Abbott Architect	Abbott Prism	BioMerieux VIDAS Duo Ultra	BioRad Access	BioRad Genscreen	DiaSorin Murex HIV	Roche Elecsys PT	Siemens ADVIA CHIV	Siemens Enzygnost Integr. II	Siemens Enzygnost Integral 4	Alere Determine	BioMerieux VIDAS p24 II	BioRad Genscreen	Innogenetics Innotest	Alliance Perkin Elmer	Roche Elecsys
pBV20-A1	3	2	2	2	2	3	3	3	2	3	0	1	3	3	3	3
pBV22-A1	3	2	3	0	2	2	3	2	2	3	0	2	3	3	2	3
pBV23-A1	3	2	3	3	3	3	3	3	2	3	0	3	3	3	3	3
pBV26-A1	3	2	2	2	2	2	2	2	2	3	0	2	3	3	3	3
pBV48-A1	3	2	3	1	3	3	3	3	3	3	0	2	3	3	3	3
pBV6-B	3	2	3	2	3	2	3	3	3	3	0	3	3	3	3	3
pBV8-B	3	3	3	1	2	2	2	2	0	1	1	2	3	0	3	3
pBV11-B	3	3	3	2	3	3	3	3	3	3	1	3	3	3	3	3
pBV15-B	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
pBV21-B	3	2	3	2	3	2	3	3	3	3	1	3	3	3	3	3
pBV25-B	3	2	3	1	2	2	3	2	3	3	1	3	3	3	3	3
pBV34-B	3	2	3	2	3	3	3	3	3	3	2	3	3	3	3	3
pBV14-C	3	3	1	3	3	2	3	2	2	3	0	0	3	3	3	3
pBV27-C	3	3	3	3	3	2	3	3	2	3	0	2	3	3	3	3
pBV31-C	3	3	3	0	2	2	3	3	2	3	0	3	3	3	3	3
pBV35-C	3	3	3	0	2	3	3	3	2	3	2	3	3	3	3	3
pBV19-C	3	3	3	3	3	3	3	3	3	3	0	3	3	3	3	3
pBV38-D	3	2	3	2	3	2	3	2	3	2	1	3	3	3	3	3
pBV63-D	2	2	2	2	2	2	2	2	2	2	1	2	2	3	3	3
pBV43-D	3	2	0	1	3	2	2	0	3	3	0	0	3	3	3	3
pBV60-D	3	2	0	0	2	2	2	1	2	3	0	0	3	2	2	3
pBV46-Bfrec	3	3	3	0	3	2	3	3	3	3	0	3	3	3	3	3
pBV55-12BF	3	3	3	3	3	2	3	2	3	3	0	3	3	3	3	3
pBV29-12BF	3	2	3	0	3	3	3	3	3	3	0	3	3	3	3	3
pBV42-12BF	3	3	1	0	3	2	2	0	3	3	0	1	3	3	3	3
pBV59-F2	3	2	3	2	3	2	2	2	0	1	0	2	3	1	3	3
pBV53-G	3	2	3	1	3	2	3	2	3	3	0	3	3	3	3	3
pBV61-G	3	2	2	0	2	2	2	2	1	2	0	2	2	2	3	3
pBV37-G/43_02G	3	3	1	1	2	3	3	3	2	3	0	1	3	3	3	3
pBV52-G/43_02G	2	2	2	0	2	2	2	2	1	2	0	2	2	2	2	2
pBV30-20BG	3	2	3	0	3	2	3	3	3	3	0	3	3	3	3	3
pBV28-01AE	3	2	3	2	3	2	3	2	3	3	0	3	3	3	3	3
pBV56-01AE	3	2	3	1	3	2	3	2	3	3	0	3	3	3	3	3
pBV12-01AE	3	2	3	1	2	2	2	2	3	3	0	2	3	3	3	3
pBV18-01AE	3	2	3	0	2	2	3	2	2	3	0	2	3	3	3	3
pBV10-02AG	2	2	2	0	2	2	2	2	2	3	0	2	2	3	3	3
pBV13-02AG	3	2	3	1	2	2	3	3	3	3	0	3	3	3	3	3
pBV17-02AG	3	2	3	0	2	2	3	2	3	3	0	3	2	3	3	3
pBV32-02AG	3	2	3	2	2	2	3	2	2	3	1	3	2	3	3	3
pBV58-02AG	3	2	2	1	2	2	2	2	0	2	0	1	2	3	3	2
pBV64-H	3	2	2	2	2	2	2	2	2	2	0	1	2	2	3	3
pBV39-O	3	2	3	0	1	2	3	2	3	3	0	2	0	3	1	3
pBV51-O	3	3	3	0	2	3	3	3	3	3	0	3	2	3	2	3
WHO	3	2	3	3	3	3	3	2	3	3	2	3	3	3	3	3
# of VLPs ND	3	30	20	77	23	32	13	30	29	9	116	32	12	9	7	1
# of VLPs D	126	99	109	52	106	97	116	99	100	119	13	97	117	120	122	128
% sensitivity	97.7	76.7	84.5	40.3	82.2	75.2	89.9	76.7	77.5	93.0	10.1	75.2	90.7	93.0	94.6	99.2

Numbers in the table indicate how many input concentrations were detected per VLP and test:

- 3 50, 10 and 2 IU/ml detected
- 2 50 and 10 IU/ml detected
- 1 50 IU/ml detected
- 0 VLP not detected at any concentration by this test

ND = not detected; D = detected

Anti-p24-antibodies

VLP-coated ELISA

VLPs were lysed, heat-denatured and cooated on 384-well plates over night. Purified antibodies (*) were tested at 5, 1 and 0.2 ug/ml concentrations, supernatants and pooled human plasma at dilutions of 1:10, 1:50 and 1:250. Primary antibodies were detected with species-specific HRP-coupled secondary antibodies. VLPs were scored as detected if the S/Co ratio was ≥2 (cut-off = no VLPs coated).

- 3 S/Co ≥2 for all antibody concentrations
- 2 S/Co ≥2 for the two higher antibody concentrations
- 1 S/Co ≥2 only for the highest antibody concentration
- 0 no detection of VLP by this antibody

		pBV20-A1	pBV22-A1	pBV23-A1	pBV26-A1	pBV48-A1	pBV6-B	pBV8-B	pBV11-B	pBV15-B	pBV21-B	pBV25-B	pBV34-B	pBV14-C	pBV27-C	pBV31-C	pBV35-C	pBV19-C	pBV38-D	pBV63-D	pBV43-D	pBV60-D	pBV46-BFrec	pBV55-12BF	pBV29-12BF	pBV42-12BF	pBV59-F2	pBV53-G	pBV61-G	pBV37-G/43_02G	pBV52-G/43_02G	pBV30-20BG	pBV28-01AE	pBV56-01AE	pBV12-01AE	pBV18-01AE	pBV10-02AG	pBV13-02AG	pBV17-02AG	pBV32-02AG	pBV58-02AG	pBV64-H	pBV39-O	pBV51-O
monoclonal	BDI690*	2	2	2	0	1	2	2	3	1	0	0	1	1	2	2	2	2	1	0	3	0	3	2	2	3	2	2	1	2	0	2	2	2	2	3	0	0	0	0	2	2	3	3
	YDHIVgp24 *	2	0	2	2	2	3	2	3	2	2	2	2	2	2	2	2	2	2	1	0	0	3	0	2	0	2	2	1	2	0	2	2	2	2	2	0	2	0	1	1	2	3	3
	N29 *	3	2	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	2	1	2	2	3	2	3	2	2	0	3	0	1	0	3	0	0
	BC1071 *	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	LH104-E	0	0	0	0	0	1	1	2	1	1	0	2	0	0	0	0	0	1	0	2	0	1	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	2	2
	AG3.0	3	2	3	2	0	0	0	3	1	0	0	1	0	2	2	2	2	2	0	3	0	3	3	3	3	0	1	0	2	0	2	2	3	2	3	0	0	0	0	2	1	3	3
	3D3	0	0	0	0	0	3	2	3	3	2	2	3	2	1	2	0	0	0	1	0	0	3	3	0	3	3	0	0	2	0	3	1	0	0	0	0	0	0	0	0	0	0	0
poly-clonal	S142	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	3	2	3	2	2	1	2	2	3	3	3
	D7320 *	2	2	3	3	3	2	3	3	3	3	2	3	2	3	2	2	2	3	3	3	3	3	3	2	3	3	2	0	3	1	3	2	3	3	2	0	2	0	2	2	3	1	1
	plasma	2	2	2	3	2	2	3	3	2	1	2	2	1	2	3	2	2	3	0	3	0	3	3	2	3	2	2	3	2	1	2	1	3	2	3	1	2	1	1	3	2	3	3

Summary

Our HIV-1 VLP subtype panel exhibits a diversity comparable to matched subtype reference sequences from the LANL database. Evaluation of commercially available HIV-1 antibodies by VLP-ELISA indicates that antibody sensitivity is determined by the methodology employed and by individual epitope variation,

Our HIV-1 Gag subtype panel served as a useful tool to assess the breadth of p24 detection. Moreover, initial results indicate that there is a need to carefully assess the capability of existing diagnostic tests for detecting p24 of diverging subtypes or with minor sequence variations.