SUPPORTING INFORMATION FOR:

Antibody Conjugation Approach Enhances Breadth and Potency of Neutralization of anti-HIV-1 Antibodies and CD4-IgG

running title: Potent, Broadly Active Bispecific anti-HIV Antibodies

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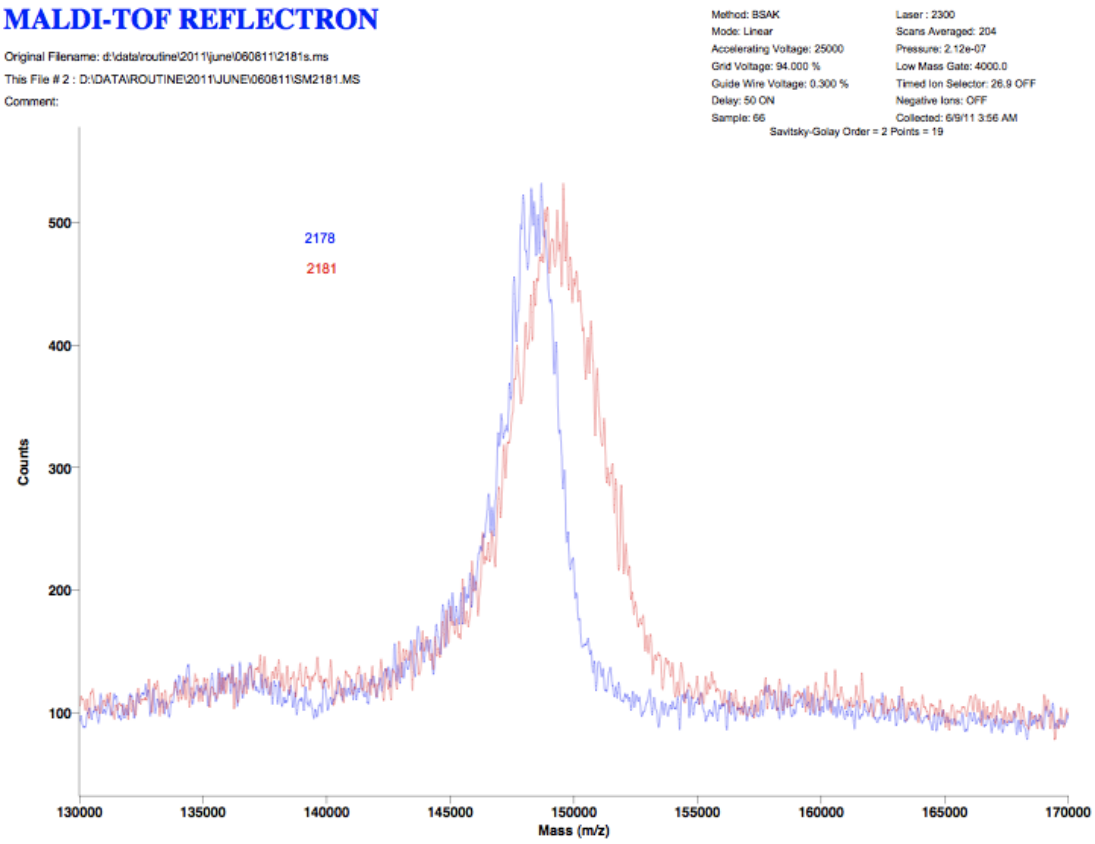
1. MALDI-TOF characterization of antibody conjugates.

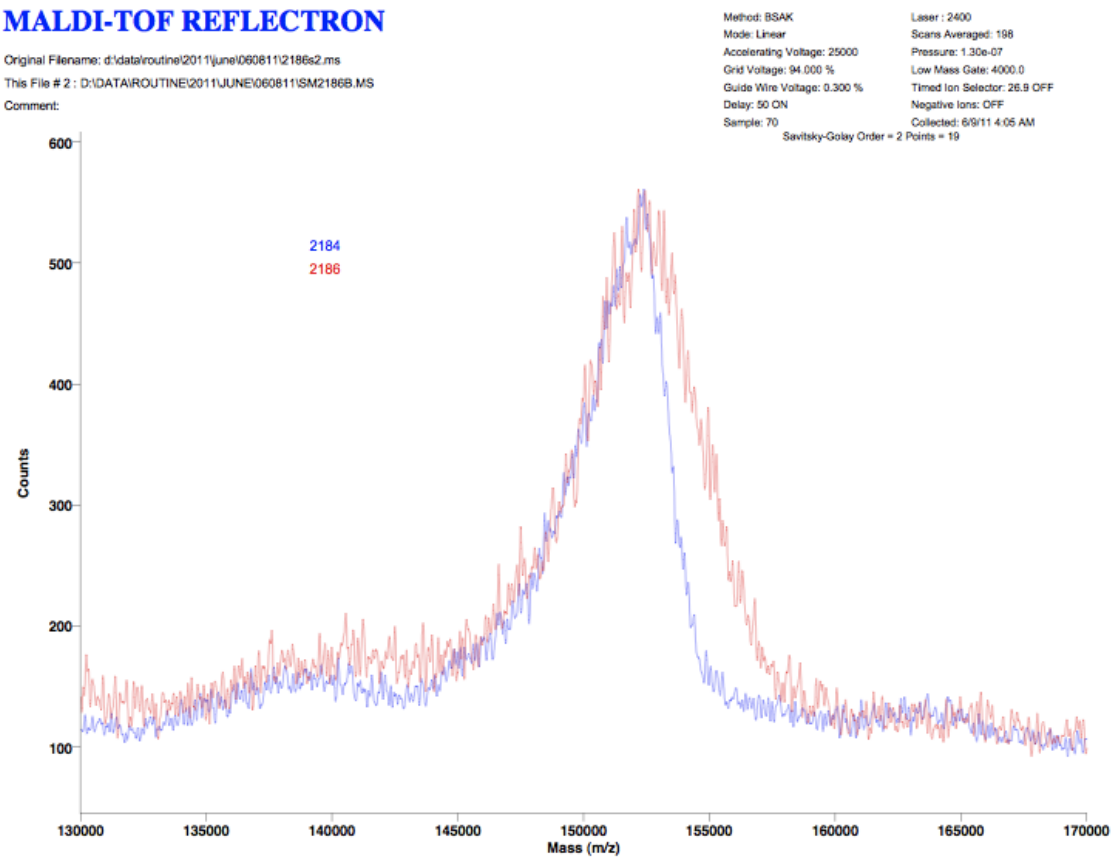
Trastuzumab-aplaviroc:

M_{av} (trastuzumab) = 148348

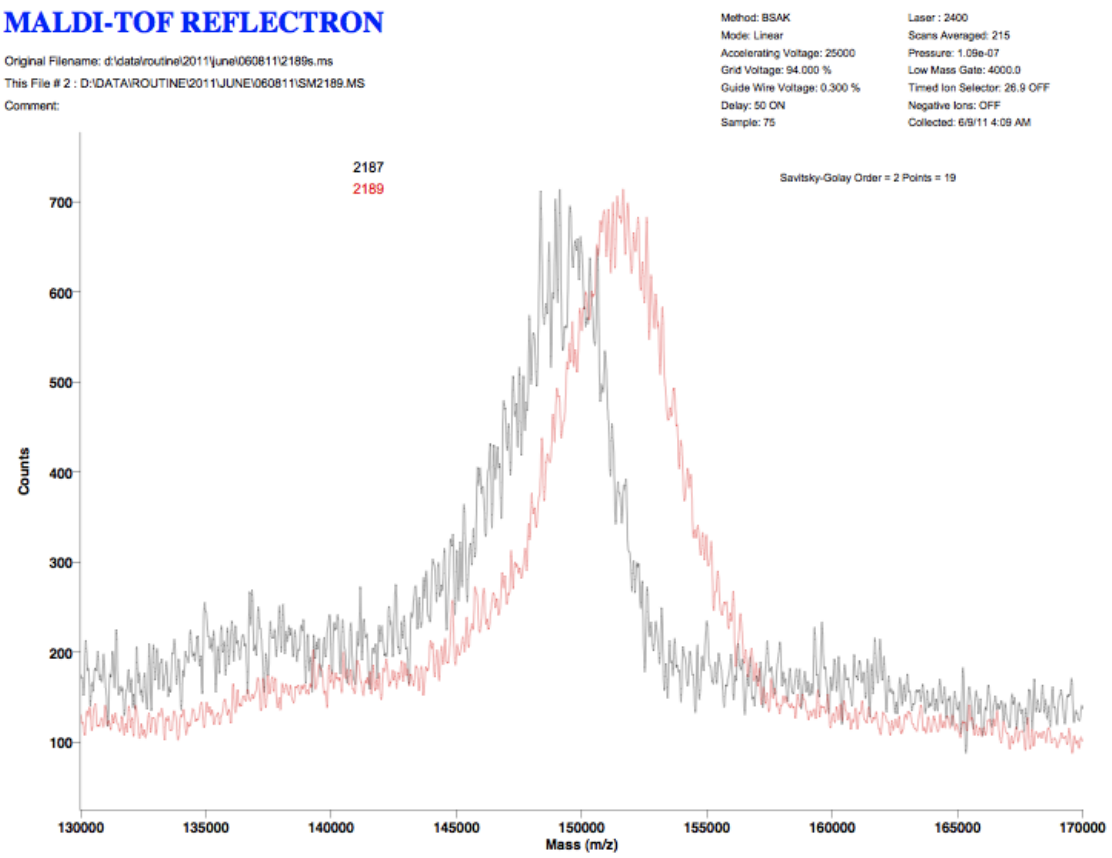
M_{av} (trastuzumab-apl) = 149243



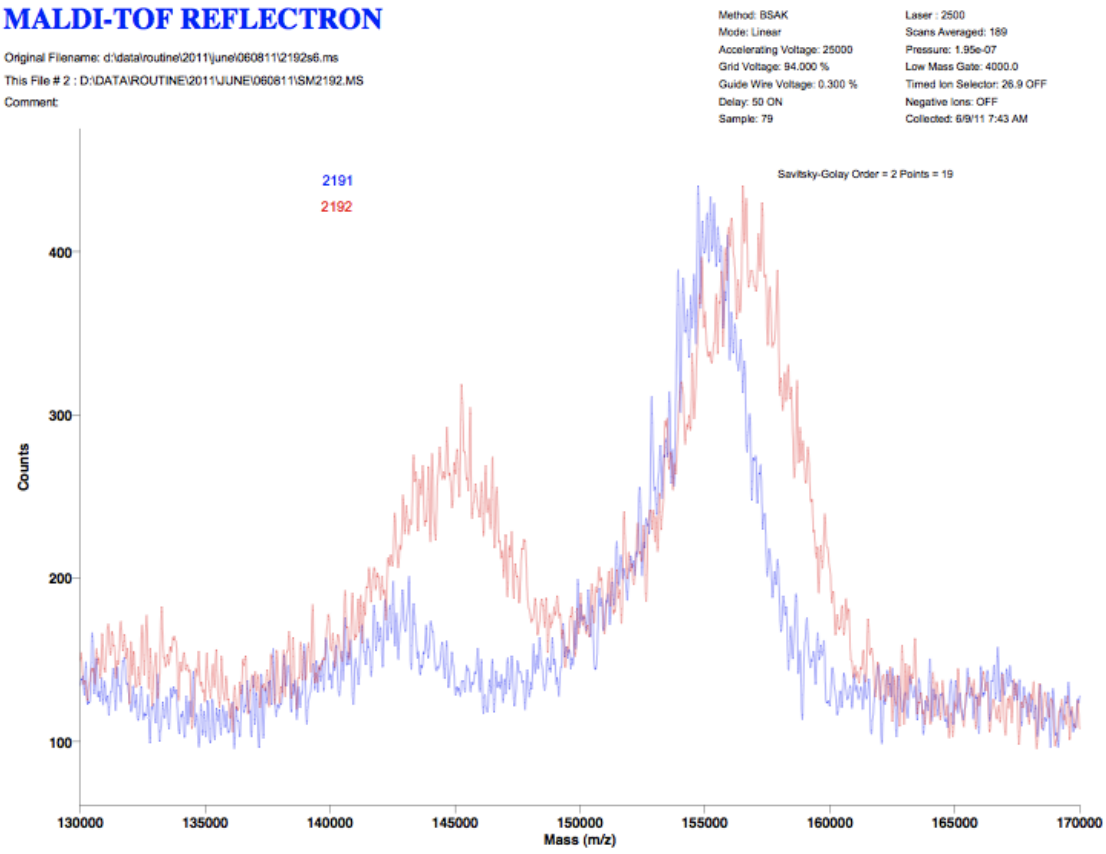
IgG b12-aplaviroc:
M_{av} (b12) = 151960
M_{av} (b12-apl) = 152347



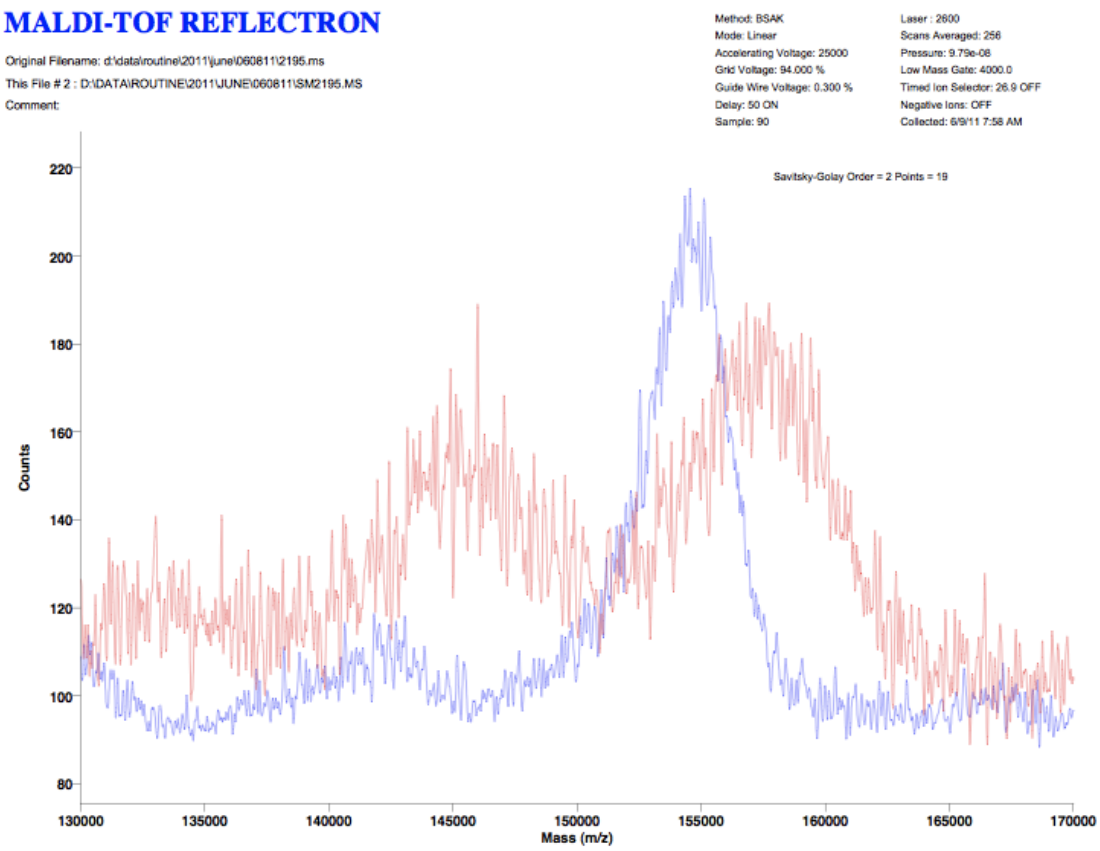
IgG 2G12-aplaviroc:
M_{av} (2G12) = 149397
M_{av} (2G12-apl) = 151464



IgG PG9-aplaviroc:
M_{av} (PG9) = 155168
M_{av} (PG9-apl) = 156408



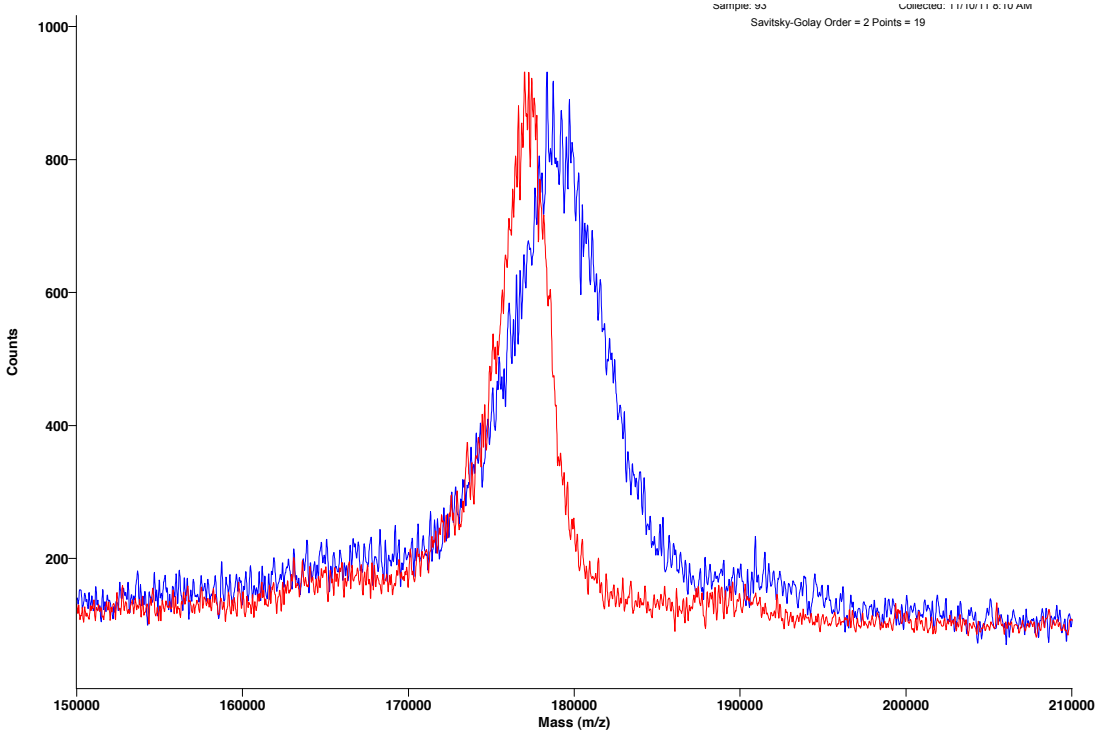
IgG PG16-aplaviroc:
M_{av} (PG16) = 154644
M_{av} (PG16-apl) = 157377



CD4-IgG-aplaviroc:

M_{av} (CD4-IgG) = 177125

M_{av} (CD4-IgG-apl) = 178900



2. Tryptic digest and MS/MS characterization of antibody conjugates.

Tryptic digest procedure.

Purified antibody samples (100 µl, 1 mg/ml) were exchanged into 6 M guanidine, 0.1 M Tris, pH 8.0 using 0.5-ml Zeba spin desalting columns (7k MWCC) as per the manufacturer's instructions. To each sample, 1 M DTT (Fisher) was added to a final concentration of 20 mM. The samples were incubated for one hour at 37°C with gentle shaking, and a 1 M solution of iodoacetamide (Fisher) was added to each sample to a final concentration of 40 mM. Samples were incubated at room temperature in the dark for 40 min. The alkylation reactions were quenched by adding 1 M DTT to a 40 mM final concentration. Sample buffer was then exchanged to trypsin digestion buffer (50 mM Tris, pH 7.5, 5 mM $CaCl_2$) using 0.5-ml Zeba spin desalting columns (7k MWCC). Trypsin Gold (0.5 mg/ml, Pierce) was dissolved in 50 mM acetic acid. Trypsin solution was added to each sample at a 1:20 ratio (enzyme:protein, weight/weight), and samples were incubated at 37ºC with shaking at 600 rpm for approximately 18 hours. Digestion was stopped by addition of TFA to approximately 0.1%.

Samples (20 µg protein injected) were analyzed using an Agilent 6510 Q-TOF mass spectrometer with Zorbax SB-C18 narrow-bore, 2.1x150 mm, 3.5-µm column (Agilent). HPLC parameters were as follows: flow rate 0.2 ml/min, gradient from 0 to 40% mobile phase B over 80 min followed by a gradient to 0% B from 80 to 90 min. Mobile phase A was 0.05% TFA (v/v) in HPLC-grade H_2O, 2% acetonitrile (v/v), and mobile phase B was 0.04% TFA (v/v) in 90% acetonitrile (v/v). MS data was collected for 200-2500 m/z and 100-2000m/z, positive polarity, gas temperature 325 °C, nebulizer 30 psi, capillary voltage 3500 V. Data was analyzed using MassHunter Software (Agilent) and GPMAW Version 8.20 Software (ChemSW).

Analysis summary.

Analysis was done using published antibody sequences.

Non-modified antibody samples were also digested and analyzed as controls (data not shown).

MassHunter Bioconfirm workflow was used with the following specifications:

“Find by molecular feature” was run with the Isotope Model “Unbiased” and limiting charged state to a maximum of 5. Allowed ion species included positive ions +H, +Na; negative ions –H; neutral loss H_2O. Other parameters were left at default values.

Aplaviroc-FBDP modification was added to the chemical modifications library and systematically applied to sequence and searched for the target modified peptide fragments. All cysteine residues were modified with iodoacetamide.

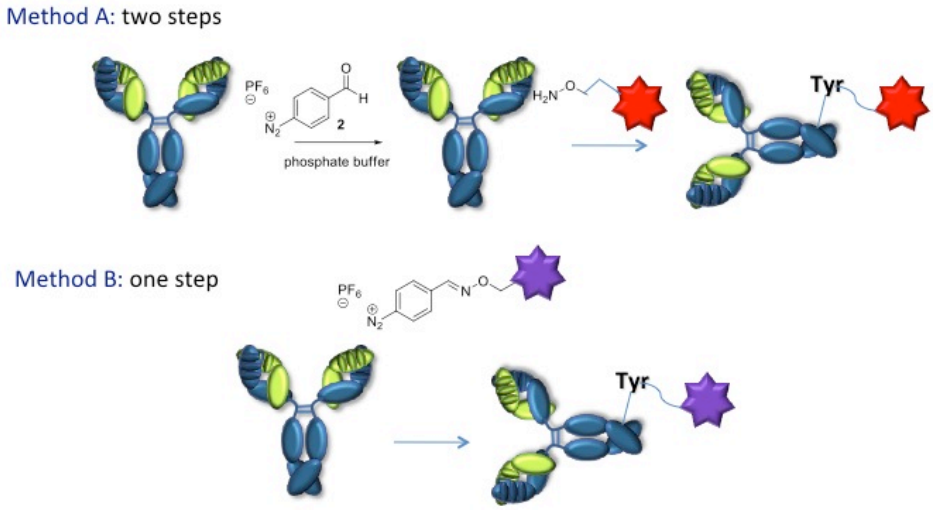
Structures of the found aplaviroc-modified fragments were confirmed by MSMS fragmentation pattern, reference MSMS fragmentation ions was calculated using GPMAW.

Table1. Summary of the identified modification positions.

Antibody	# Sammples tested	Identified Fragment Sequence	Peptide fragment Expected Mass:MH+, M2H+, M3H+	Found Mass	Position modified	Average Signal Intensity
Trastuzumab	4	NTAYLQMNSLR	2405.232, 1203.120, 802.416	1203.1, 802.4	Y80 (heavy chain)	1.20E+03
Trastuzumab	4	EEQYNSTYR	2284.092, 1142.550, 762.036	1142.55, 762.0	Y300 (heavy chain)	5.30E+04
b12	2	VSCQASGYR	2064.013, 1032.510, 688.676	1032.5, 688.7	Y27 (heavy chain)	3.10E+03
b12	2	EEQYNSTYR	2284.092, 1142.550, 762.036	1142.6, 762.0	Y306 (heavy chain)	6.01E+04
2g12	2	EEQYNSTYR	2284.092, 1142.550, 762.036	1142.6, 762.0	Y404(heavy chain)	4.70E+04
2g12	2	WQQGNVFSCSVMHEALHNHYTQ	3864.841, 1932.924, 1288.952	1289	Y544(heavy chain)	1.80E+04
2g12	2	SGTASVVCLLNNFYPR	2861.469, 1431.238, 954.495	1431.2, 954.5	Y139 (light chain)	8.20E+02
PG9	2	EEQYNSTYR	2284.092, 1142.550, 762.036	1142.55, 762.0	Y314(heavy chain)	3.40E+04
PG9	2	SGTASVVCLLNNFYPR	2861.469, 1431.238, 954.495	1431.2, 954.5	Y143(light chain)	9.50E+02
PG16	2	YHSDSMWGR	2233.054, 1117.030, 745.023	1117.03, 745.0	Y59(heavy chain)	2.50E+02
PG16	2	EEQYNSTYR	2284.092, 1142.550, 762.036	1142.55, 762.0	Y413(heavy chain)	3.10E+03
PG16	2	EYK	1533.799, 767.403, 511.938	1533.8, 767.4	Y436(heavy chain)	1.20E+02

3. Optimization studies for the modification of model antibody (Trastuzumab).

Optimization study was performed with trastuzumab as model IgG in order to identify optimal procedure for antibody modification, assess reproducibility and protein recovery.



General protocol for the two-step procedure.

Step 1: To the 200 μL of trastuzumab solution (1.5 mg/ml, 0.1M Na_2HPO_4 solution pH 8.0) was added FBDP solution in CH_3CN (2 μL of 20 mM solution). Reaction mixture was vortexed gently and allowed to react for 30 min. Reaction turned yellow. The reaction was run through the gel filtration spin column to remove excess FBDP and exchange buffer to 0.1M phosphate buffer pH 6.0. Results summarized in Table 2.

Step 2: To the solution of FBDP modified antibody from step 1 were added 4 μL of 20 mM solution of aplaviroc-oxyamine, reaction was vortexed and allowed to react at 4°C for 18h. The resulting conjugate was purified using gel filtration spin column, 20 μL stacker used. Results summarized in Table 3.

Protein concentration in the resulting solution was determined using two independent methods. One method based on the absorbance at 280 nm (Nanodrop) with the correction for the absorbance of diazene product at 340 nm, correction factor of 0.436. Trastuzumab extinction coefficient was calculated using trastuzumab sequence and ExPasy ProtParam tool (http://web.expasy.org/cgi-bin/protparam/protparam). Another was based on the

Bradford test for protein concentration, using unmodified trastuzumab solution of known concentration as standard.

Table 2. Summary of the first step experiments in two-step antibody modification procedure.

#	Her conc., μM	FBDP eq	Precipitation observed	Column for gel filtration	Recovery, %	Comments
1	6.7	10	No	Bio-Rad	69	Phosphate buffer pH 7.4
2	6.7	10	No	Bio-Rad	66	Phosphate buffer pH 8.0
3	6.7	10	No	Bio-Rad	69	Phosphate buffer pH 7.4 + 0.1M urea
4	100	10	No	Bio-Rad	98	Phosphate buffer pH 8.0
5	10	10	No	Bio-Rad	13	Phosphate buffer pH 8.0
6	10	10	No	Bio-Rad	35	Phosphate buffer pH 8.0
7	10	10	No	Zeba	65	Phosphate buffer pH 8.0
8	10	10	No	Zeba	60	Phosphate buffer pH 8.0
9	17	10	No	Zeba	99	Phosphate buffer pH 8.0
10	10	10	No	Bio-Rad	83	Phosphate buffer pH 8.0
11	10	10	No	Zeba	99	Phosphate buffer pH 8.0
12	10	10	No	Bio-Rad	77	Phosphate buffer pH 8.0
13	10	10	No	Zeba	99	Phosphate buffer pH 8.0
14	10	10	No	Bio-Rad	84	Phosphate buffer pH 8.0
15	10	10	No	Zeba	99	Phosphate buffer pH 8.0

Table 3. Summary of the second step experiments in two-step antibody modification procedure.

#	Aplaviroc-ONH_2 eq.	Column for gel filtration	Recovery, %	Average # of modifications/Ab	Comments
1	**29**	**Bio-Rad**	**50**	**0.96**	**o/n, 4°C**
2	**29**	**Bio-Rad**	**54**	**0.69**	**o/n, 4°C**
3	**29**	**Bio-Rad**	**56**	**0.85**	**o/n, 4°C**
4	**20**	**Bio-Rad**	**99**	**1.56**	**o/n, 4°C**

5	**20**	**Bio-Rad**	**40**	**-**	**o/n, 4°C**
6	**20**	**Bio-Rad**	**30**	**-**	**o/n, 4°C**
7	**20**	**Zeba**	**87**	**2.25**	**o/n, 4°C**
8	**20**	**Zeba**	**73**	**2.5**	**o/n, 4°C**
9	**10**	**Zeba**	**99**	**2.13**	**o/n, 4°C**
10	**20**	**Bio-Rad**	**58**	**1.54**	**o/n, 4°C**
11	**20**	**Zeba**	**71**	**2.07**	**o/n, 4°C**
12	**20**	**Bio-Rad**	**62**	**-**	**o/n, 4°C**
13	**20**	**Zeba**	**71**	**-**	**o/n, 4C**
14	**20**	**Bio-Rad**	**57**	**-**	**o/n, 4C**
15	**20**	**Zeba**	**71**	**2.03**	**o/n, 4C**

General protocol for the one-step antibody modification procedure.

In 150 μL eppendorf tube were combined 1.6 μL of 100 mM acid solution in CH_3CN and 4 μL of 20 mM aplaviroc-oxyamine followed by 4 μL of 20 mM FBDP in CH_3CN and mixed, allowed to preform the oxime at rt for **30** min. Then, 200 μL of trastuzumab solution (1.5 mg/ml, 0.1M Na_2HPO_4 buffer pH 8.0) were added, vortexed gently and allowed to react for 30 min at rt. Reaction mixture turned yellow. Reaction mixture was run through the gel filtration spin column to remove excess of the reagents, 10 μL stacker used. Results summarized in Tables 4 and 5.

Protein concentration in the resulting solution was determined using two independent methods. One method based on the absorbance at 280 nm (Nanodrop) with the correction for the absorbance of diazene product at 340 nm, correction factor of 0.436. Trastuzumab extinction coefficient was calculated using trastuzumab sequence and ExPasy ProtParam tool (http://web.expasy.org/cgi-bin/protparam/protparam). Another was based on the Bradford test for protein concentration, using unmodified trastuzumab solution of known concentration as standard.

Table 4. Summary of the optimization experiments in one-step antibody modification procedure at pH 8.0.

#	Acid Additive	Column for gel filtration	Recovery, %	# modifications/Ab	Precipitation observed
1	-	Bio-Rad	64	0.36	no
2	AcOH	Bio-Rad	68	0.3	no
3	HCl	Bio-Rad	65	0	no
4	TFA	Zeba	97	0.47	yes
5	TFA	Zeba	99	0.28	yes
6	TFA	Zeba	45	0.39	yes
7	HCl	Zeba	99	0.51	yes
8	AcOH	Zeba	99	0.21	yes
9	TFA	Zeba	95	0.52	yes
10	HCl	Zeba	99	0.41	yes
11	AcOH	Zeba	99	0.14	yes
12	TFA	Zeba	99	0.31	yes

Table 5. Summary of the optimization experiments in one-step antibody modification procedure at pH 9.0.

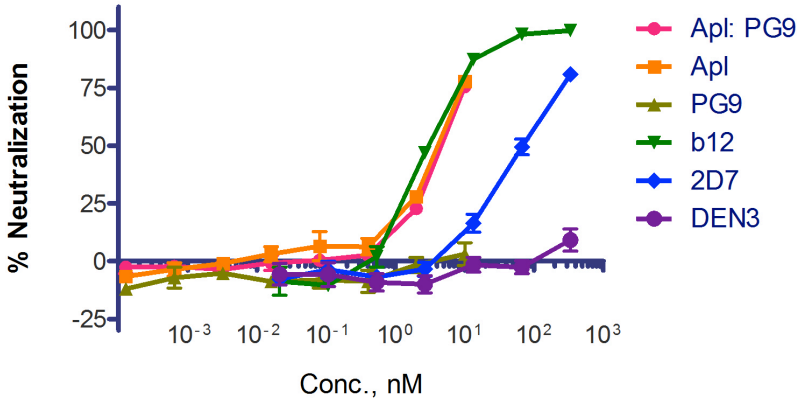
#	Acid additive	Column for gel filtration	% recovery	# modifications/Ab
1	TFA	Zeba	82	0.44
2	H_2SO_4 (aq)	Zeba	99	-
3	H_3PO_4 (conc).	Zeba	95	0.46
4	TFA	Zeba	91	0.56
5	HCl	Zeba	99	1.95
6	H_2SO_4	Zeba	86	0.39
7	H_3PO_4	Zeba	99	1

8	HCl	Bio-Rad	71	0.7
9	H_3PO_4	Bio-Rad	75	0.9
10	HCl	Bio-Rad	75	0
11	H_3PO_4	Bio-Rad	57	0.2
12	HCl	Bio-Rad	71	0.6
13	H_3PO_4	Bio-Rad	75	1
14	HCl	Zeba	76	0.3
15	H_3PO_4	Zeba	99	1
16	HCl	Zeba	99	0.4
17	HCl	Zeba	97	0.82
18	H_3PO_4	Zeba	87	1.54
19	H_3PO_4	Zeba	86	0.84

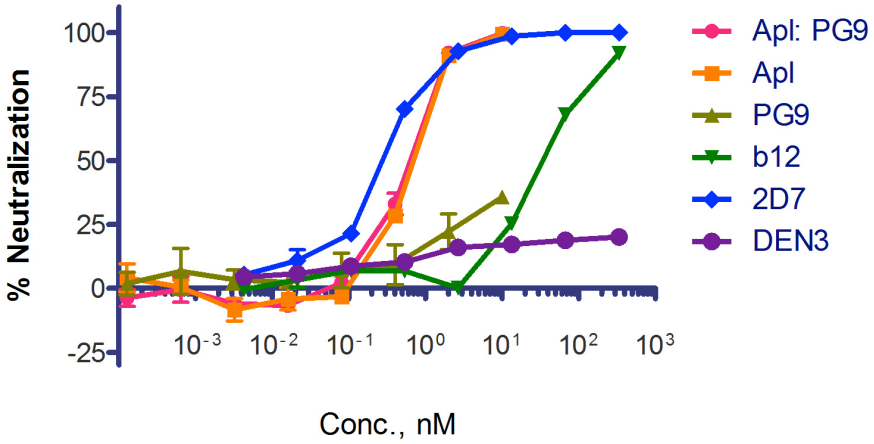
4. Additional Neutralization Experiments.

Neutralization of the CCR5-tropic HIV-1 JR-FL and YU2 by 1:1 molar mixture PG9 and Aplaviroc.

Neutralization of HIV-1 JR-FL Pseudovirus

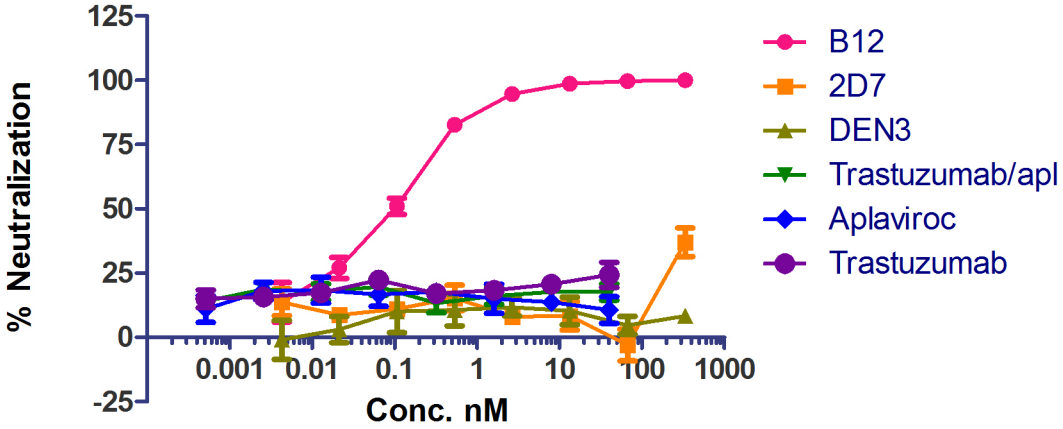


Neutralization of HIV-1 YU2 Pseudovirus



Neutralization of the CXCR4-tropic HIV-1 HXB2 virus.

Neutralization of HIV-1 HXB2 Pseudovirus



5.Neutralization Results for 117 Pseudovirus Panel Screen.

IC50s for the full panel.

Virus ID	Clade*	IC50 Titer in TZM-bl cells, µg/ml		IC50 Titer in TZM-bl cells, nM		
		PG9**	PG9/aplaviroc	Aplaviroc (nM)	PG9**	PG9/aplaviroc
6535.3	B	0.176	0.494	1.021	1.173	3.294
QH0692.42	B	>50	10.817	17.438	>333	72.112
SC422661.8	B	0.553	2.279	7.309	3.687	15.193
PVO.4	B	10.564	0.716	0.805	70.427	4.773
TRO.11	B	43.247	6.544	19.277	288.313	43.624
AC10.0.29	B	0.117	0.192	25.034	0.78	1.277
RHPA4259.7	B	22.620	2.035	2.843	150.8	13.569
THRO4156.18	B	24.475	4.856	11.205	163.167	32.376
REJO4541.67	B	0.012	0.020	21.009	0.08	0.134
TRJO4551.58	B	0.503	1.120	25.481	3.353	7.468
WITO4160.33	B	0.010	0.012	4.593	0.067	0.078
CAAN5342.A2	B	4.603	4.701	6.255	30.687	31.339
Median		0.553	1.578	9.257	3.687	10.518
Mean		9.716	2.815	11.856	64.776	18.770
STD		14.408	3.327	9.319	96.053	22.181
WEAU_d15_410_5017	B (T/F)	4.078	1.087	100.000	27.187	7.250
1006_11_C3_1601	B (T/F)	0.366	0.466	4.086	2.44	3.109
1054_07_TC4_1499	B (T/F)	>50	6.916	7.873	>333	46.107
1056_10_TA11_1826	B (T/F)	6.339	4.513	6.238	42.26	30.084
1012_11_TC21_3257	B (T/F)	0.172	0.470	21.247	1.147	3.132
6240_08_TA5_4622	B (T/F)	1.747	2.998	14.962	11.647	19.989
6244_13_B5_4576	B (T/F)	>50	7.674	9.738	>333	51.160
62357_14_D3_4589	B (T/F)	>50	4.305	4.156	>333	28.700
SC05_8C11_2344	B (T/F)	0.892	1.385	5.663	5.947	9.233
Median		1.320	2.998	7.873	8.797	19.989
Mean		2.266	3.313	19.329	15.105	22.085
STD		2.450	2.724	30.770	16.337	18.163
Du156.12	C	0.026	0.088	17.108	0.173	0.588
Du172.17	C	0.606	0.581	10.435	4.04	3.871
Du422.1	C	0.614	0.512	10.456	4.093	3.412
ZM197M.PB7	C	0.899	0.442	2.343	5.993	2.947
ZM214M.PL15	C	>50	2.136	2.419	>333	14.241
ZM233M.PB6	C	0.010	0.003	0.847	0.067	0.023
ZM249M.PL1	C	0.166	0.110	0.817	1.107	0.734
ZM53M.PB12	C	0.069	0.112	0.656	0.46	0.745
ZM109F.PB4	C	0.256	0.309	1.325	1.707	2.061
ZM135M.PL10a	C	20.559	5.023	5.045	137.06	33.485
CAP45.2.00.G3	C	0.007	0.003	0.693	0.0467	0.017
CAP210.2.00.E8	C	0.040	0.261	15.878	0.267	1.737
HIV-001428-2.42	C	0.101	0.003	4.599	0.673	0.017
HIV-0013095-2.11	C	0.010	0.039	0.947	0.067	0.263
HIV-16055-2.3	C	0.021	0.022	1.781	0.14	0.145
HIV-16845-2.22	C	4.398	4.444	5.539	29.32	29.624
Median		0.101	0.186	2.381	0.673	1.241
Mean		1.852	0.880	5.056	12.348	5.869
STD		5.293	1.594	5.482	35.287	10.629
Ce1086_B2	C (T/F)	>50	1.183	1.145	>333	7.888
Ce0393_C3	C (T/F)	0.020	0.024	0.330	0.133	0.157
Ce1176_A3	C (T/F)	0.014	0.034	14.886	0.093	0.224
Ce2010_F5	C (T/F)	>50	11.145	19.793	>333	74.297
Ce0682_E4	C (T/F)	0.196	0.208	1.901	1.307	1.384
Ce1172_H1	C (T/F)	0.070	0.106	6.919	0.467	0.706
Ce2060_G9	C (T/F)	0.045	0.104	4.183	0.3	0.695
Ce703010054_2A2	C (T/F)	0.022	0.014	0.394	0.147	0.095
BF1266.431a	C (T/F)	0.026	0.080	1.471	0.173	0.532
246F C1G	C (T/F)	>50	1.262	1.734	>333	8.414
249M B10	C (T/F)	0.095	0.129	0.699	0.633	0.863
ZM247v1(Rev-)	C (T/F)	0.112	0.138	0.891	0.747	0.918
7030102001E5(Rev-)	C (T/F)	>50	6.763	5.871	>333	45.087
1394C9G1(Rev-)	C (T/F)	0.029	0.043	2.306	0.193	0.286
Ce704809221_1B3	C (T/F)	0.037	0.201	12.650	0.247	1.339
Median		0.037	0.129	1.901	0.247	0.863
Mean		0.061	1.429	5.012	0.404	9.526
STD		0.061	1.429	5.012	0.404	9.526
CNE19	BC	0.024	0.055	2.444	0.16	0.364
CNE20	BC	0.052	0.182	1.694	0.347	1.216
CNE21	BC	0.038	0.112	0.748	0.253	0.745
CNE17	BC	0.210	0.332	5.516	1.4	2.213
CNE30	BC	>50	9.645	11.919	>333	64.297
CNE52	BC	0.029	0.176	>100	0.193	1.176
CNE53	BC	0.131	0.700	18.158	0.873	4.666
CNE58	BC	0.038	0.229	20.777	0.253	1.524
Median		0.038	0.205	5.516	0.253	1.370
Mean		0.075	1.429	8.751	0.497	9.525
STD		0.070	3.326	8.239	0.466	22.171
MS208.A1	A	0.005	0.110	0.202	0.033	0.734
Q23.17	A	0.027	0.013	13.939	0.18	0.090
Q461.e2	A	1.484	1.470	3.681	9.893	9.798
Q769.d22	A	0.025	0.020	3.424	0.167	0.134
Q259.d2.17	A	0.029	0.140	10.726	0.193	0.935
Q842.d12	A	0.023	0.058	37.578	0.153	0.387
0330.v4.c3	A	0.026	0.035	2.390	0.173	0.235
0260.v5.c36	A	1.693	1.866	17.280	11.287	12.437
Median		0.0265	0.084	7.2035	0.1765	0.560
Mean		0.414	0.464	11.153	2.760	3.094
STD		0.727	0.752	12.283	4.847	5.011

IC50s continued:

191955_A11	A (T/F)	0.053	0.017	0.032	0.353	0.112
191084 B7-19	A (T/F)	0.043	0.091	4.471	0.287	0.605
9004SS_A3_4	A (T/F)	0.088	0.130	5.825	0.587	0.868
Median		0.053	0.091	4.471	0.353	0.605
Mean		0.061	0.079	3.443	0.409	0.528
STD		0.024	0.058	3.030	0.158	0.384
T257-31	CRF02_AG	0.037	0.089	16.777	0.247	0.594
928-28	CRF02_AG	0.101	0.213	9.155	0.673	1.423
263-8	CRF02_AG	0.293	0.541	4.779	1.953	3.608
T250-4	CRF02_AG	0.005	0.001	14.339	0.033	0.007
T251-18	CRF02_AG	>50	16.982	22.188	>333	113.210
T278-50	CRF02_AG	0.393	1.484	9.074	2.62	9.893
T255-34	CRF02_AG	0.023	0.048	6.700	0.153	0.319
211-9	CRF02_AG	0.049	0.215	19.148	0.327	1.434
235-47	CRF02_AG	0.233	0.531	1.075	1.553	3.540
Median		0.075	0.215	9.155	0.5	1.434
Mean		0.142	2.234	11.471	0.945	14.892
STD		0.146	5.549	7.044	0.971	36.994
620345.c01	CRF01_AE	1.333	2.278	6.946	8.887	15.187
C1080.c03	CRF01_AE	0.004	0.006	25.834	0.0267	0.039
R2184.c04	CRF01_AE	0.255	0.666	5.517	1.7	4.443
R1166.c01	CRF01_AE	0.736	1.108	4.644	4.907	7.389
R3265.c06	CRF01_AE	0.163	0.423	3.334	1.087	2.818
C2101.c01	CRF01_AE	0.034	0.032	1.832	0.227	0.213
C3347.c11	CRF01_AE	0.032	0.176	1.756	0.213	1.176
C4118.c09	CRF01_AE	0.068	0.071	0.603	0.453	0.476
CNE5	CRF01_AE	0.018	0.020	1.186	0.12	0.134
BJOX009000.02.4	CRF01_AE	1.725	2.957	3.915	11.5	19.714
Median		0.116	0.300	3.625	0.770	1.997
Mean		0.437	0.774	5.557	2.912	5.159
STD		0.622	1.045	7.405	4.149	6.969
BJOX015000.11.5	CRF01_AE (T/F)	0.322	0.748	2.991	2.147	4.986
BJOX010000.06.2	CRF01_AE (T/F)	0.154	0.603	1.529	1.027	4.023
BJOX025000.01.1	CRF01_AE (T/F)	0.144	0.309	0.436	0.96	2.061
BJOX028000.10.3	CRF01_AE (T/F)	1.100	0.597	0.372	7.333	3.977
Median		0.238	0.600	0.983	1.587	4.000
Mean		0.430	0.564	1.332	2.867	3.762
STD		0.454	0.184	1.227	3.027	1.225
X1193_c1	G	0.105	0.355	2.076	0.7	2.364
P0402_c2_11	G	0.296	0.317	1.341	1.973	2.112
X1254_c3	G	0.065	0.311	3.047	0.433	2.073
X2088_c9	G	>50	10.453	12.647	>333	69.687
X2131_C1_B5	G	0.084	0.511	3.621	0.56	3.406
P1981_C5_3	G	0.258	1.062	25.451	1.72	7.082
X1632_S2_B10	G	0.107	0.681	6.005	0.713	4.538
Median		0.106	0.511	3.621	0.7065	3.406
Mean		0.153	1.956	7.741	1.017	13.037
STD		0.098	3.757	8.687	0.656	25.044
3016.v5.c45	D	2.455	0.409	0.394	16.367	2.729
A07412M1.vrc12	D	0.697	1.296	9.851	4.647	8.639
231965.c01	D	1.285	1.943	5.641	8.567	12.952
231966.c02	D	0.075	0.107	4.337	0.5	0.712
Median		0.991	0.853	4.989	6.607	5.684
Mean		1.128	0.939	5.056	7.520	6.258
STD		1.013	0.838	3.898	6.755	5.589
191821_E6_1	D (T/F)	3.600	0.313	0.188	24	2.084
Median(D+D(T/F))		1.285	0.409	4.337	8.567	2.729
Mean(D+D(T/F))		1.622	0.813	4.082	10.816	5.423
STD(D+D(T/F))		1.411	0.778	4.017	9.410	5.187
3817.v2.c59	CD	0.020	0.003	4.230	0.133	0.017
6480.v4.c25	CD	>50	1.476	1.378	>333	9.843
6952.v1.c20	CD	>50	1.666	1.642	>333	11.109
6811.v7.c18	CD	>50	5.080	6.954	>333	33.866
89-F1_2_25	CD	0.668	0.587	0.573	4.453	3.916
Median		0.344	1.476	1.642	2.293	9.843
Mean		0.344	1.763	2.955	2.293	11.750
STD		0.458	1.973	2.622	3.055	13.155
3301.v1.c24	AC	0.216	0.445	4.349	1.44	2.969
6041.v3.c23	AC	0.244	0.627	3.498	1.627	4.179
6540.v4.c1	AC	0.070	0.116	13.148	0.467	0.773
6545.v4.c1	AC	0.099	0.287	4.814	0.66	1.910
Median		0.158	0.366	4.582	1.050	2.439
Mean		0.157	0.369	6.452	1.049	2.458
STD		0.086	0.218	4.497	0.571	1.456
0815.v3.c3	ACD	>50	1.399	1.532	>333	9.328
3103.v3.c10	ACD	28.004	27.728	63.328	186.693	184.851
Median		28.004	14.563	32.430	186.693	97.089
Mean		28.004	14.563	32.430	186.693	97.089
STD			18.617	43.696		124.114

Note: Calculation of Mean and Median omits any values with “>” sign.

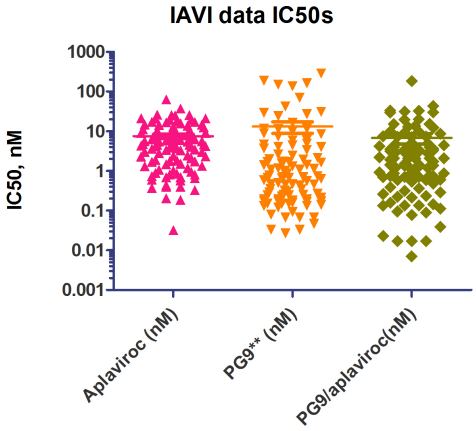


Figure 1. Graphical representation of the IC50s for all samples. PG9 IC50s>333nM are omitted for clarity.

Statistical analysis for a set of pseudoviruses, where PG9 IC50<50 ug/ml.

The following Mean and Median IC50s were calculated by GraphPad Prism analysis:

PG9 Median IC50 = 0.713nM, Mean IC50 =1.91nM

PG9/aplaviroc Median IC50 = 13.1nM, Mean IC50 = 6.79nM

Aplaviroc Median IC50 =4.35nM, Mean IC50 =7.56nM.

Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC50 values of PG9 and PG9/aplaviroc (P value 0.0547).

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC50 values of Aplaviroc and PG9/aplaviroc (P value 0.0002).

Pseudoviruses where PG9-aplaviroc has improved IC50 relative to wildtype PG9. Pseudoviruses neutralized by PG9 with IC50>50 μg/ml (>333nM) are grouped separately.

Improved IC50s for PG9/apl vs PG9

Virus ID	Clade*	IC50 Titer in TZM-bl cells, μg/ml		IC50 Titer in TZM-bl cells, nM		
		PG9**	PG9/aplaviroc	Aplaviroc (nM)	PG9**	PG9/aplaviroc
PVO.4	B	10.564	0.716	0.805	70.427	4.773
TRO.11	B	43.247	6.544	19.277	288.313	43.624
RHPA4259.7	B	22.620	2.035	2.843	150.8	13.569
THRO4156.18	B	24.475	4.856	11.205	163.167	32.376
1056_10_TA11_1826	B (T/F)	6.339	4.513	6.238	42.26	30.084
Du172.17	C	0.606	0.581	10.435	4.04	3.871
Du422.1	C	0.614	0.512	10.456	4.093	3.412
ZM197M.PB7	C	0.899	0.442	2.343	5.993	2.947
ZM233M.PB6	C	0.010	0.003	0.847	0.067	0.023
ZM249M.PL1	C	0.166	0.110	0.817	1.107	0.734
HIV-001428-2.42	C	0.101	0.003	4.599	0.673	0.017
Ce703010054_2A2	C (T/F)	0.022	0.014	0.394	0.147	0.095
Q23.17	A	0.027	0.013	13.939	0.18	0.090
Q461.e2	A	1.484	1.470	3.681	9.893	9.798
Q769.d22	A	0.025	0.020	3.424	0.167	0.134
191955_A11	A (T/F)	0.053	0.017	0.032	0.353	0.112
T250-4	CRF02_AG	0.005	0.001	14.339	0.033	0.007
C2101.c01	CRF01_AE	0.034	0.032	1.832	0.227	0.213
3016.v5.c45	D	2.455	0.409	0.394	16.367	2.729
191821_E6_1	D (T/F)	3.600	0.313	0.188	24	2.084
3817.v2.c59	CD	0.020	0.003	4.230	0.133	0.017
89-F1_2_25	CD	0.668	0.587	0.573	4.453	3.916
3103.v3.c10	ACD	28.004	27.728	63.328	186.693	184.851
Median		0.614	0.409	3.424	4.093	2.729
Mean		6.349	2.214	7.662	42.330	14.760
STD		11.703	5.843	13.312	78.018	38.953

Average improvement of 2.87 fold relative to PG9
Average decrease of potency relative to aplaviroc: 1.93 fold

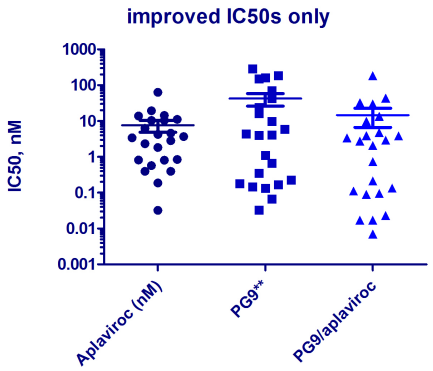


Figure 2. Graphical representation of the improved IC50s. Pseudoviruses where PG9 has IC50>50 μg/ml are grouped separately.

Statistical analysis for a set of pseudoviruses, where PG9/aplaviroc has improved IC50s relative to PG9.

The following Mean and Median IC50s were calculated by GraphPad Prism analysis:

PG9 Median IC50 = 4.09nM, Mean IC50 = 42.33nM

PG9/aplaviroc Median IC50 = 2.73nM, Mean IC50 = 14.76nM

Aplaviroc Median IC50 =3.42nM, Mean IC50 =7.66nM.

Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC50 values of PG9 and PG9/aplaviroc (P value 0.1533).

Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC50 values of Aplaviroc and PG9/aplaviroc (P value 0.3018).

Pseudoviruses neutralized by PG9 with IC50>50 μg/ml (>333nM):

Improved from IC50 >50 ug/ml

Virus ID	Clade*	IC50 Titer in TZM-bl cells, μg/ml		IC50 Titer in TZM-bl cells, nM		
		PG9**	PG9/aplaviroc	Aplaviroc (nM)	PG9**	PG9/aplaviroc
QH0692.42	B	>50	10.817	17.438	>333	72.112
1054_07_TC4_1499	B (T/F)	>50	6.916	7.873	>333	46.107
6244_13_B5_4576	B (T/F)	>50	7.674	9.738	>333	51.160
62357_14_D3_4589	B (T/F)	>50	4.305	4.156	>333	28.700
ZM214M.PL15	C	>50	2.136	2.419	>333	14.241
Ce1086_B2	C (T/F)	>50	1.183	1.145	>333	7.888
Ce2010_F5	C (T/F)	>50	11.145	19.793	>333	74.297
246F C1G	C (T/F)	>50	1.262	1.734	>333	8.414
7030102001E5(Rev-)	C (T/F)	>50	6.763	5.871	>333	45.087
CNE30	BC	>50	9.645	11.919	>333	64.297
T251-18	CRF02_AG	>50	16.982	22.188	>333	113.210
X2088_c9	G	>50	10.453	12.647	>333	69.687
6480.v4.c25	CD	>50	1.476	1.378	>333	9.843
6952.v1.c20	CD	>50	1.666	1.642	>333	11.109
6811.v7.c18	CD	>50	5.080	6.954	>333	33.866
0815.v3.c3	ACD	>50	1.399	1.532	>333	9.328
Median		>50	5.921	6.413	>333	39.476
Mean		>50	6.181	8.027	>333	41.209
STD			4.703	6.992		31.354

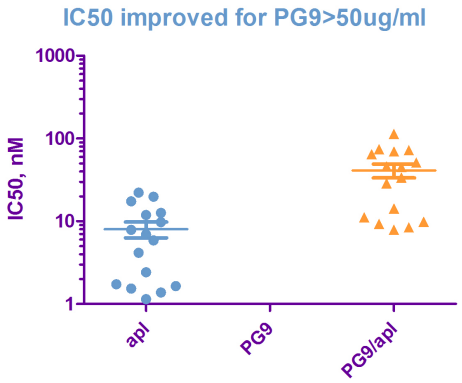


Figure 3. Graphical representation of the IC50s of aplaviroc and PG9-aplaviroc in the panel of pseudoviruses poorly neutralized by PG9 (IC50>50 μg/ml).

Statistical analysis for a set of pseudoviruses, where PG9 IC50>50ug/ml.

The following Mean and Median IC50s were calculated by GraphPad Prism analysis:

PG9/aplaviroc Median IC50 = 39.48nM, Mean IC50 = 41.21nM

Aplaviroc Median IC50 =6.41nM, Mean IC50 =8.03nM.

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC50 values of Aplaviroc and PG9/aplaviroc (P value 0.0003).

Pseudoviruses where PG9-aplaviroc shows decreased potency (IC50) relative to wildtype PG9:

Decrease of potency of PG9/apl relative to PG9

Virus ID	Clade*	IC50 Titer in TZM-bl cells, µg/ml		IC50 Titer in TZM-bl cells, nM		
		PG9**	PG9/aplaviroc	Aplaviroc (nM)	PG9**	PG9/aplaviroc
6535.3	B	0.176	0.494	1.021	1.173	3.294
SC422661.8	B	0.553	2.279	7.309	3.687	15.193
AC10.0.29	B	0.117	0.192	25.034	0.78	1.277
REJO4541.67	B	0.012	0.020	21.009	0.08	0.134
TRJO4551.58	B	0.503	1.120	25.481	3.353	7.468
WITO4160.33	B	0.010	0.012	4.593	0.067	0.078
CAAN5342.A2	B	4.603	4.701	6.255	30.687	31.339
WEAU_d15_410_5017	B (T/F)	4.078	1.087	>100	27.187	7.250
1006_11_C3_1601	B (T/F)	0.366	0.466	4.086	2.44	3.109
1012_11_TC21_3257	B (T/F)	0.172	0.470	21.247	1.147	3.132
6240_08_TA5_4622	B (T/F)	1.747	2.998	14.962	11.647	19.989
SC05_8C11_2344	B (T/F)	0.892	1.385	5.663	5.947	9.233
Du156.12	C	0.026	0.088	17.108	0.173	0.588
ZM53M.PB12	C	0.069	0.112	0.656	0.46	0.745
ZM109F.PB4	C	0.256	0.309	1.325	1.707	2.061
CAP210.2.00.E8	C	0.040	0.261	15.878	0.267	1.737
HIV-0013095-2.11	C	0.010	0.039	0.947	0.067	0.263
HIV-16055-2.3	C	0.021	0.022	1.781	0.14	0.145
HIV-16845-2.22	C	4.398	4.444	5.539	29.32	29.624
Ce0393_C3	C (T/F)	0.020	0.024	0.330	0.133	0.157
Ce1176_A3	C (T/F)	0.014	0.034	14.886	0.093	0.224
Ce0682_E4	C (T/F)	0.196	0.208	1.901	1.307	1.384
Ce1172_H1	C (T/F)	0.070	0.106	6.919	0.467	0.706
Ce2060_G9	C (T/F)	0.045	0.104	4.183	0.3	0.695
249M B10	C (T/F)	0.095	0.129	0.699	0.633	0.863
ZM247v1(Rev-)	C (T/F)	0.112	0.138	0.891	0.747	0.918
1394C9G1(Rev-)	C (T/F)	0.029	0.043	2.306	0.193	0.286
Ce704809221_1B3	C (T/F)	0.037	0.201	12.650	0.247	1.339
CNE19	BC	0.024	0.055	2.444	0.16	0.364
CNE20	BC	0.052	0.182	1.694	0.347	1.216
CNE21	BC	0.038	0.112	0.748	0.253	0.745
CNE17	BC	0.210	0.332	5.516	1.4	2.213
CNE52	BC	0.029	0.176	>100	0.193	1.176
CNE53	BC	0.131	0.700	18.158	0.873	4.666
CNE58	BC	0.038	0.229	20.777	0.253	1.524
MS208.A1	A	0.005	0.110	0.202	0.033	0.734
Q259.d2.17	A	0.029	0.140	10.726	0.193	0.935
Q842.d12	A	0.023	0.058	37.578	0.153	0.387
0330.v4.c3	A	0.026	0.035	2.390	0.173	0.235
0260.v5.c36	A	1.693	1.866	17.280	11.287	12.437
191084 B7-19	A (T/F)	0.043	0.091	4.471	0.287	0.605
9004SS_A3_4	A (T/F)	0.088	0.130	5.825	0.587	0.868
T257-31	CRF02_AG	0.037	0.089	16.777	0.247	0.594
928-28	CRF02_AG	0.101	0.213	9.155	0.673	1.423
263-8	CRF02_AG	0.293	0.541	4.779	1.953	3.608
T278-50	CRF02_AG	0.393	1.484	9.074	2.62	9.893
T255-34	CRF02_AG	0.023	0.048	6.700	0.153	0.319
211-9	CRF02_AG	0.049	0.215	19.148	0.327	1.434
235-47	CRF02_AG	0.233	0.531	1.075	1.553	3.540
620345.c01	CRF01_AE	1.333	2.278	6.946	8.887	15.187
C1080.c03	CRF01_AE	0.004	0.006	25.834	0.0267	0.039
R2184.c04	CRF01_AE	0.255	0.666	5.517	1.7	4.443
R1166.c01	CRF01_AE	0.736	1.108	4.644	4.907	7.389
R3265.c06	CRF01_AE	0.163	0.423	3.334	1.087	2.818
C3347.c11	CRF01_AE	0.032	0.176	1.756	0.213	1.176
C4118.c09	CRF01_AE	0.068	0.071	0.603	0.453	0.476
CNE5	CRF01_AE	0.018	0.020	1.186	0.12	0.134
BJOX009000.02.4	CRF01_AE	1.725	2.957	3.915	11.5	19.714
BJOX015000.11.5	CRF01_AE (T/F)	0.322	0.748	2.991	2.147	4.986

Decreased potency (IC50) continued on the next page.

BJOX010000.06.2	CRF01_AE (T/F)	0.154	0.603		1.529	1.027	4.023
BJOX025000.01.1	CRF01_AE (T/F)	0.144	0.309		0.436	0.96	2.061
BJOX028000.10.3	CRF01_AE (T/F)	1.100	0.597		0.372	7.333	3.977
X1193_c1	G	0.105	0.355		2.076	0.7	2.364
P0402_c2_11	G	0.296	0.317		1.341	1.973	2.112
X1254_c3	G	0.065	0.311		3.047	0.433	2.073
X2131_C1_B5	G	0.084	0.511		3.621	0.56	3.406
P1981_C5_3	G	0.258	1.062		25.451	1.72	7.082
X1632_S2_B10	G	0.107	0.681		6.005	0.713	4.538
A07412M1.vrc12	D	0.697	1.296		9.851	4.647	8.639
231965.c01	D	1.285	1.943		5.641	8.567	12.952
231966.c02	D	0.075	0.107		4.337	0.5	0.712
3301.v1.c24	AC	0.216	0.445		4.349	1.44	2.969
6041.v3.c23	AC	0.244	0.627		3.498	1.627	4.179
6540.v4.c1	AC	0.070	0.116		13.148	0.467	0.773
6545.v4.c1	AC	0.099	0.287		4.814	0.66	1.910
Median		0.101	0.261		4.644	0.673	1.737
Mean		0.425	0.625		7.745	2.834	4.164
STD		0.907	0.936		8.065	6.044	6.241

Average decrease of potency of PG9/apl relative to PG9: 1.47 fold
Average improvement of potency for PG9/apl relative to aplaviroc: 2.45 fold

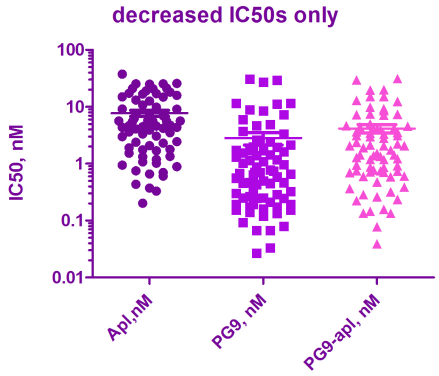


Figure 4. Graphical representation of IC50s in the pseudovirus panel where PG9-aplaviroc has decreased potency relative to PG9.

Statistical analysis for a set of pseudoviruses, where PG9/aplaviroc has decreased potency relative to PG9.

The following Mean and Median IC50s were calculated by GraphPad Prism analysis:

PG9 Median IC50 = 0.67nM, Mean IC50 = 2.73nM

PG9/aplaviroc Median IC50 = 1.74nM, Mean IC50 = 4.16nM

Aplaviroc Median IC50 =4.64nM, Mean IC50 =7.75nM.

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC50 values of PG9 and PG9/aplaviroc (P value 0.0012).

Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC50 values of Aplaviroc and PG9/aplaviroc (P value <0.0001).

IC80s for the full panel.

		IC80 Titer in TZM-bl cells, µg/ml			IC80 Titer in TZM-bl cells, nM		
Virus ID	Clade*	PG9*	PG9/aplaviroc		Aplaviroc (nM)	PG9*	PG9/aplaviroc
6535.3	B	0.627	1.490		12.517	4.18	9.933
QH0692.42	B	>50	>50		98.769	>333	>333
SC422661.8	B	7.054	12.585		79.320	47.027	83.899
PVO.4	B	>50	8.239		11.173	>333	54.930
TRO.11	B	>50	35.584		100.000	>333	237.227
AC10.0.29	B	0.845	1.810		87.686	5.633	12.067
RHPA4259.7	B	>50	7.092		19.358	>333	47.277
THRO4156.18	B	>50	20.071		61.850	>333	133.804
REJO4541.67	B	0.163	0.185		82.676	1.087	1.233
TRJO4551.58	B	4.061	8.795		100.000	27.073	58.633
WITO4160.33	B	0.037	0.049		78.852	0.247	0.325
CAAN5342.A2	B	26.992	13.870		30.832	179.947	92.465
Median		0.845	8.239		79.086	5.633	54.930
Mean		5.683	9.979		63.586	37.885	66.527
STD		9.746	10.609		35.295	64.973	70.726
WEAU_d15_410_5017	B (T/F)	>50	19.772		>100	>333	131.815
1006_11_C3_1601	B (T/F)	1.906	2.119		32.098	12.707	14.129
1054_07_TC4_1499	B (T/F)	>50	36.094		57.473	>333	240.628
1056_10_TA11_1826	B (T/F)	38.259	23.408		66.049	255.06	156.056
1012_11_TC21_3257	B (T/F)	0.605	4.402		98.066	4.034	29.345
6240_08_TA5_4622	B (T/F)	6.333	10.408		67.658	42.22	69.384
6244_13_B5_4576	B (T/F)	>50	28.640		46.026	>333	190.935
62357_14_D3_4589	B (T/F)	>50	17.254		16.826	>333	115.025
SC05_8C11_2344	B (T/F)	4.283	6.784		32.815	28.553	45.227
Median		4.283	17.254		51.750	28.553	115.025
Mean		10.277	16.542		52.126	68.515	110.283
STD		15.796	11.592		25.722	105.309	77.280
Du156.12	C	0.190	0.342		93.800	1.267	2.280
Du172.17	C	1.604	2.746		62.373	10.693	18.308
Du422.1	C	10.398	2.824		52.130	69.32	18.824
ZM197M.PB7	C	4.517	2.214		13.654	30.113	14.762
ZM214M.PL15	C	>50	18.933		40.579	>333	126.218
ZM233M.PB6	C	0.026	0.030		3.294	0.173	0.202
ZM249M.PL1	C	1.410	0.409		4.246	9.4	2.729
ZM53M.PB12	C	0.221	0.509		6.009	1.473	3.395
ZM109F.PB4	C	6.689	2.457		10.570	44.593	16.381
ZM135M.PL10a	C	>50	21.958		21.910	>333	146.387
CAP45.2.00.G3	C	0.029	0.006		15.920	0.193	0.039
CAP210.2.00.E8	C	0.173	3.241		78.371	1.153	21.608
HIV-001428-2.42	C	0.250	0.027		28.984	1.667	0.179
HIV-0013095-2.11	C	0.030	0.170		7.069	0.2	1.132
HIV-16055-2.3	C	0.06	0.110		8.758	0.4	0.734
HIV-16845-2.22	C	>50	18.415		31.308	>333	122.767
Median		0.221	1.362		18.915	1.473	9.079
Mean		1.969	4.649		29.936	13.127	30.996
STD		3.261	7.622		28.166	21.740	50.812
Ce1086_B2	C (T/F)	>50	4.069		4.462	>333	27.126
Ce0393_C3	C (T/F)	0.055	0.084		2.152	0.367	0.561
Ce1176_A3	C (T/F)	0.044	0.117		87.151	0.293	0.779
Ce2010_F5	C (T/F)	>50	>50		>100	>333	333.000
Ce0682_E4	C (T/F)	0.698	1.507		29.311	4.653	10.045
Ce1172_H1	C (T/F)	0.206	0.530		74.695	1.373	3.535
Ce2060_G9	C (T/F)	0.172	0.297		17.488	1.147	1.983
Ce703010054_2A2	C (T/F)	0.103	0.089		3.355	0.687	0.594
BF1266.431a	C (T/F)	0.088	0.192		5.441	0.587	1.283
246F C1G	C (T/F)	>50	3.500		8.549	>333	23.333
249M B10	C (T/F)	0.290	0.508		2.674	1.933	3.384
ZM247v1(Rev-)	C (T/F)	0.323	0.565		8.042	2.153	3.765
7030102001E5(Rev-)	C (T/F)	>50	29.579		20.305	>333	197.193
1394C9G1(Rev-)	C (T/F)	0.114	0.155		12.912	0.760	1.036
Ce704809221_1B3	C (T/F)	0.137	1.233		69.147	0.913	8.218
Median		0.137	0.519		10.731	0.913	3.535
Mean		0.203	3.030		24.692	1.352	41.056
STD		0.203	3.030		24.692	1.352	41.056
CNE19	BC	0.146	0.245		17.066	0.973	1.630
CNE20	BC	0.278	0.637		8.431	1.853	4.246
CNE21	BC	0.094	0.392		5.283	0.627	2.611
CNE17	BC	0.484	1.171		29.677	3.227	7.810
CNE30	BC	>50	41.902		39.067	>333	279.345
CNE52	BC	0.081	0.821		100.000	0.540	5.473
CNE53	BC	0.60	3.285		60.248	3.967	21.899
CNE58	BC	0.122	0.732		51.858	0.813	4.880
Median		0.146	0.776		34.372	0.973	5.176
Mean		0.257	6.148		38.954	1.714	40.987
STD		0.206	14.478		31.571	1.372	96.522
MS208.A1	A	0.014	0.409		1.099	0.093	2.728
Q23.17	A	0.082	0.052		95.241	0.547	0.347
Q461.e2	A	8.725	5.678		21.460	58.167	37.854
Q769.d22	A	0.068	0.099		28.769	0.453	0.661
Q259.d2.17	A	1.379	1.247		48.616	9.193	8.314
Q842.d12	A	0.081	0.215		>100	0.540	1.434
0330.v4.c3	A	0.076	0.159		8.747	0.507	1.059
0260.v5.c36	A	9.0	10.948		97.906	59.953	72.986
Median		0.1	0.312		28.769	0.543	2.081
Mean		2.427	2.351		43.120	16.182	15.673
STD		3.996	3.959		39.519	26.642	26.390

IC80s continued:

191955_A11	A (T/F)	0.154	0.069		0.236	1.027	0.459
191084 B7-19	A (T/F)	0.202	0.346		26.672	1.347	2.308
9004SS_A3_4	A (T/F)	0.251	0.484		24.314	1.673	3.227
Median		0.202	0.346		24.314	1.347	2.308
Mean		0.202	0.300		17.074	1.349	1.998
STD		0.049	0.211		14.630	0.323	1.410
T257-31	CRF02_AG	0.124	0.353		68.083	0.827	2.353
928-28	CRF02_AG	0.450	1.587		59.885	3.000	10.577
263-8	CRF02_AG	1.050	1.784		44.140	7.000	11.894
T250-4	CRF02_AG	0.014	0.005		44.660	0.093	0.034
T251-18	CRF02_AG	>50	>50		94.866	>333	>333
T278-50	CRF02_AG	3.192	5.504		43.820	21.280	36.695
T255-34	CRF02_AG	0.134	0.364		27.550	0.893	2.426
211-9	CRF02_AG	0.243	0.834		94.925	1.620	5.563
235-47	CRF02_AG	1.201	1.882		8.140	8.007	12.543
Median		0.347	1.211		44.660	2.310	8.070
Mean		0.801	1.539		54.008	5.340	10.261
STD		1.062	1.754		28.872	7.081	11.694
620345.c01	CRF01_AE	>50	14.409		33.074	>333	96.062
C1080.c03	CRF01_AE	0.013	0.024		82.338	0.087	0.162
R2184.c04	CRF01_AE	1.237	3.136		21.270	8.247	20.908
R1166.c01	CRF01_AE	2.876	4.213		27.921	19.173	28.090
R3265.c06	CRF01_AE	0.535	1.408		22.063	3.567	9.384
C2101.c01	CRF01_AE	0.099	0.191		10.347	0.660	1.272
C3347.c11	CRF01_AE	0.113	1.065		13.964	0.753	7.098
C4118.c09	CRF01_AE	0.200	0.262		3.051	1.333	1.748
CNE5	CRF01_AE	0.051	0.061		3.470	0.340	0.409
BJOX009000.02.4	CRF01_AE	8.211	11.028		35.617	54.740	73.518
Median		0.200	1.236		21.667	1.333	8.241
Mean		1.482	3.580		25.312	9.878	23.865
STD		2.688	5.076		23.040	17.922	33.841
BJOX015000.11.5	CRF01_AE (T/F)	1.489	5.628		45.477	9.927	37.518
BJOX010000.06.2	CRF01_AE (T/F)	0.740	2.804		19.761	4.933	18.695
BJOX025000.01.1	CRF01_AE (T/F)	0.335	2.721		18.749	2.233	18.140
BJOX028000.10.3	CRF01_AE (T/F)	21.500	4.000		17.776	143.333	26.667
Median		1.115	3.402		19.255	7.430	22.681
Mean		6.016	3.788		25.441	40.107	25.255
STD		10.334	1.358		13.382	68.892	9.056
X1193_c1	G	0.304	1.745		16.344	2.027	11.630
P0402_c2_11	G	2.367	1.821		19.745	15.780	12.140
X1254_c3	G	0.304	1.557		17.921	2.027	10.381
X2088_c9	G	>50	30.361		55.550	>333	202.403
X2131_C1_B5	G	0.286	2.383		19.084	1.907	15.888
P1981_C5_3	G	2.512	5.532		71.099	16.747	36.880
X1632_S2_B10	G	0.934	5.046		96.596	6.227	33.641
Median		0.619	2.383		19.745	4.127	15.888
Mean		1.118	6.921		42.334	7.452	46.138
STD		1.054	10.464		32.323	7.027	69.763
3016.v5.c45	D	>50	1.571		2.193	>333	10.476
A07412M1.vrc12	D	7.963	6.715		49.448	53.087	44.768
231965.c01	D	47.969	12.029		49.438	319.793	80.190
231966.c02	D	0.257	0.708		14.785	1.713	4.723
Median		7.963	4.143		32.112	53.087	27.622
Mean		18.730	5.256		28.966	124.864	35.039
STD		25.613	5.236		24.197	170.756	34.908
191821_E6_1	D (T/F)	>50	0.976		1.298	>333	6.510
Median(D+D(T/F))		7.963	1.571		14.785	53.087	10.476
Mean(D+D(T/F))		18.730	4.400		23.432	124.864	29.333
STD(D+D(T/F))		25.613	4.922		24.336	170.756	32.813
3817.v2.c59	CD	0.062	0.030		31.940	0.413	0.202
6480.v4.c25	CD	>50	8.097		10.890	>333	53.983
6952.v1.c20	CD	>50	11.544		19.843	>333	76.958
6811.v7.c18	CD	>50	24.897		50.284	>333	165.983
89-F1_2_25	CD	10.103	2.209		2.289	67.353	14.728
Median		5.083	8.097		19.843	33.883	53.983
Mean		5.083	9.356		23.049	33.883	62.371
STD		7.100	9.823		18.774	47.334	65.486
3301.v1.c24	AC	0.756	2.568		24.002	5.040	17.120
6041.v3.c23	AC	3.639	3.224		13.488	24.260	21.490
6540.v4.c1	AC	0.253	0.694		55.967	1.687	4.627
6545.v4.c1	AC	0.351	1.082		25.819	2.340	7.210
Median		0.554	1.825		24.911	3.690	12.165
Mean		1.250	1.892		29.819	8.332	12.612
STD		1.608	1.200		18.260	10.718	8.002
0815.v3.c3	ACD	>50	7.406		7.363	>333	49.373
3103.v3.c10	ACD	>50	50.000		100.000	>333	>333
Median		>50	28.703		53.682	>333	49.373
Mean		>50	28.703		53.682	>333	49.373
STD			30.119		65.504		

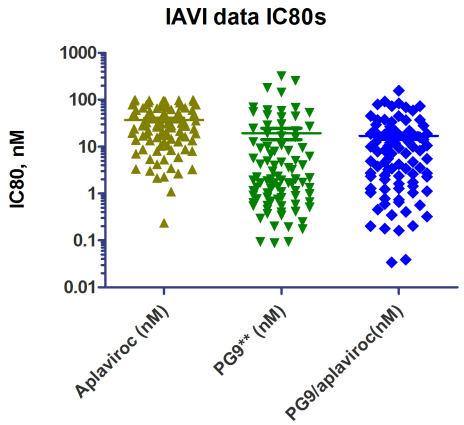


Figure 5. Graphical representation of the IC80s for all samples. PG9 IC80s>333nM are omitted for clarity.

Statistical analysis for all samples with PG9 IC80<50 ug/ml.

The following Mean and Median IC80s were calculated by GraphPad Prism analysis:

PG9 Median IC80 = 1.98nM, Mean IC80 = 19.46nM

PG9/aplaviroc Median IC80 = 7.51nM, Mean IC80 = 17.00nM

Aplaviroc Median IC80 =27.55nM, Mean IC80 =37.22nM.

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC80 values of PG9 and PG9/aplaviroc (P value 0.0161).

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC80 values of Aplaviroc and PG9/aplaviroc (P value <0.0001).

Pseudoviruses where PG9-aplaviroc has improved IC80 relative to wildtype PG9. Pseudoviruses neutralized by PG9 with IC80>50 μg/ml (>333nM) are grouped separately.

Improved IC80s

		IC80 Titer in TZM-bl cells, μg/ml			IC80 Titer in TZM-bl cells, nM		
Virus ID	Clade*	PG9*	PG9/aplaviroc		Aplaviroc (nM)	PG9*	PG9/aplaviroc
1056_10_TA11_1826	B (T/F)	38.259	23.408		66.049	255.06	156.056
Du172.17	C	1.604	2.746		62.373	10.693	18.308
Du422.1	C	10.398	2.824		52.130	69.32	18.824
ZM197M.PB7	C	4.517	2.214		13.654	30.113	14.762
ZM233M.PB6	C	0.026	0.030		3.294	0.173	0.202
ZM249M.PL1	C	1.410	0.409		4.246	9.4	2.729
HIV-001428-2.42	C	0.250	0.027		28.984	1.667	0.179
Ce703010054_2A2	C (T/F)	0.103	0.089		3.355	0.687	0.594
Q23.17	A	0.082	0.052		95.241	0.547	0.347
Q461.e2	A	8.725	5.678		21.460	58.167	37.854
Q769.d22	A	0.068	0.099		28.769	0.453	0.661
191955_A11	A (T/F)	0.154	0.069		0.236	1.027	0.459
T250-4	CRF02_AG	0.014	0.005		44.660	0.093	0.034
C2101.c01	CRF01_AE	0.099	0.191		10.347	0.660	1.272
3817.v2.c59	CD	0.062	0.030		31.940	0.413	0.202
A07412M1.vrc12	D	7.963	6.715		49.448	53.087	44.768
6041.v3.c23	AC	3.639	3.224		13.488	24.260	21.490
231965.c01	D	47.969	12.029		49.438	319.793	80.190
BJOX028000.10.3	CRF01_AE (T/F)	21.500	4.000		17.776	143.333	26.667
CAAN5342.A2	B	26.992	13.870		30.832	179.947	92.465
ZM109F.PB4	C	6.689	2.457		10.570	44.593	16.381
P0402_c2_11	G	2.367	1.821		19.745	15.780	12.140
89-F1_2_25	CD	10.103	2.209		2.289	67.353	14.728
Median		2.367	2.209		21.460	15.780	14.728
Mean		8.391	3.661		28.710	55.940	24.405
STD		13.099	5.702		24.684	87.324	38.016

Average improvement of 2.29 fold relative to PG9
Average decrease of potency relative to aplaviroc : 1.18 fold

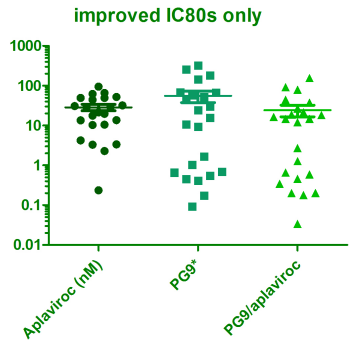


Figure 6. Graphical representation of the improved IC80s. Pseudoviruses where PG9 has IC80>50 μg/ml are grouped separately.

Statistical analysis for all samples with improved PG9/aplaviroc IC80s relative to PG9. Sample set where PG9 has IC80> 50ug/ml is analyzed separately.

The following Mean and Median IC80s were calculated by GraphPad Prism analysis:
PG9 Median IC80 = 15.78nM, Mean IC80 = 55.94nM
PG9/aplaviroc Median IC80 = 14.73nM, Mean IC80 = 24.4nM
Aplaviroc Median IC80 =21.46nM, Mean IC80 =28.71nM.
Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC80 values of PG9 and PG9/aplaviroc (P value 0.3448).

Mann Whitney test has shown that in this set of samples there is no statistically significant difference between Median IC80 values of Aplaviroc and PG9/aplaviroc (P value 0.0826).
Pseudoviruses neutralized by PG9 with IC80>50 μg/ml (>333nM):

		IC80 Titer in TZM-bl cells, μg/ml			IC80 Titer in TZM-bl cells, nM		
Virus ID	Clade*	PG9*	PG9/aplaviroc		Aplaviroc (nM)	PG9*	PG9/aplaviroc
WEAU_d15_410_5017	B (T/F)	>50	19.772		>100	>333	131.815
HIV-16845-2.22	C	>50	18.415		31.308	>333	122.767
620345.c01	CRF01_AE	>50	14.409		33.074	>333	96.062
0815.v3.c3	ACD	>50	7.406		7.363	>333	49.373
6480.v4.c25	CD	>50	8.097		10.890	>333	53.983
6952.v1.c20	CD	>50	11.544		19.843	>333	76.958
6811.v7.c18	CD	>50	24.897		50.284	>333	165.983
X2088_c9	G	>50	30.361		55.550	>333	202.403
3016.v5.c45	D	>50	1.571		2.193	>333	10.476
191821_E6_1	D (T/F)	>50	0.976		1.298	>333	6.510
246F C1G	C (T/F)	>50	3.500		8.549	>333	23.333
7030102001E5(Rev-)	C (T/F)	>50	29.579		20.305	>333	197.193
CNE30	BC	>50	41.902		39.067	>333	279.345
Ce1086_B2	C (T/F)	>50	4.069		4.462	>333	27.126
ZM214M.PL15	C	>50	18.933		40.579	>333	126.218
PVO.4	B	>50	8.239		11.173	>333	54.930
TRO.11	B	>50	35.584		100.000	>333	237.227
RHPA4259.7	B	>50	7.092		19.358	>333	47.277
THRO4156.18	B	>50	20.071		61.850	>333	133.804
1054_07_TC4_1499	B (T/F)	>50	36.094		57.473	>333	240.628
6244_13_B5_4576	B (T/F)	>50	28.640		46.026	>333	190.935
62357_14_D3_4589	B (T/F)	>50	17.254		16.826	>333	115.025
Median			17.834		20.305		118.896
Mean			17.655		30.356		117.699
STD			12.160		25.118		81.066

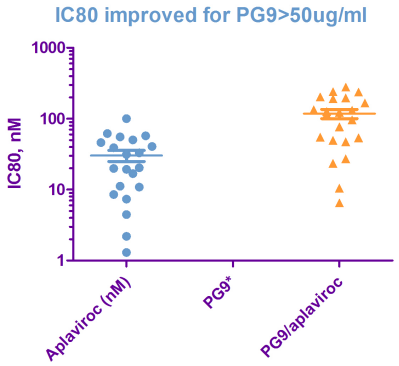


Figure 7. Graphical representation of the IC80s of aplaviroc and PG9-aplaviroc in the panel of pseudoviruses poorly neutralized by PG9 (IC80>50 μg/ml).

Statistical analysis for all samples where PG9 has IC80> 50ug/ml.
The following Mean and Median IC80s were calculated by GraphPad Prism analysis:
PG9/aplaviroc Median IC80 = 118.9nM, Mean IC80 = 117.7nM
Aplaviroc Median IC80 =20.31nM, Mean IC80 =30.36nM.
Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC80 values of Aplaviroc and PG9/aplaviroc (P value 0.0001).

Pseudoviruses neutralized by both PG9 and PG9-aplaviroc with IC80>50 μg/ml (>333nM):

		IC80 Titer in TZM-bl cells, μg/ml			IC80 Titer in TZM-bl cells, nM		
Virus ID	Clade*	PG9*	PG9/aplaviroc		Aplaviroc (nM)	PG9*	PG9/aplaviroc
3103.v3.c10	ACD	>50	>50		>100	>333	>333
T251-18	CRF02_AG	>50	>50		94.866	>333	>333
Ce2010_F5	C (T/F)	>50	>50		>100	>333	>333
QH0692.42	B	>50	>50		98.769	>333	>333

Pseudoviruses where PG9-aplaviroc shows decreased potency (IC80) relative to wildtype PG9:

Decrease of potency of PG9/apl relative to PG9, IC80s

		IC80 Titer in TZM-bl cells, μg/ml			IC80 Titer in TZM-bl cells, nM		
Virus ID	Clade*	PG9*	PG9/aplaviroc		Aplaviroc (nM)	PG9*	PG9/aplaviroc
6535.3	B	0.627	1.490		12.517	4.18	9.933
SC422661.8	B	7.054	12.585		79.320	47.027	83.899
AC10.0.29	B	0.845	1.810		87.686	5.633	12.067
REJO4541.67	B	0.163	0.185		82.676	1.087	1.233
TRJO4551.58	B	4.061	8.795		>100	27.073	58.633
WITO4160.33	B	0.037	0.049		78.852	0.247	0.325
1006_11_C3_1601	B (T/F)	1.906	2.119		32.098	12.707	14.129
1012_11_TC21_3257	B (T/F)	0.605	4.402		98.066	4.034	29.345
6240_08_TA5_4622	B (T/F)	6.333	10.408		67.658	42.22	69.384
SC05_8C11_2344	B (T/F)	4.283	6.784		32.815	28.553	45.227
Du156.12	C	0.190	0.342		93.800	1.267	2.280
ZM53M.PB12	C	0.221	0.509		6.009	1.473	3.395
CAP210.2.00.E8	C	0.173	3.241		78.371	1.153	21.608
HIV-0013095-2.11	C	0.030	0.170		7.069	0.2	1.132
HIV-16055-2.3	C	0.06	0.110		8.758	0.4	0.734
Ce0393_C3	C (T/F)	0.055	0.084		2.152	0.367	0.561
Ce1176_A3	C (T/F)	0.044	0.117		87.151	0.293	0.779
Ce0682_E4	C (T/F)	0.698	1.507		29.311	4.653	10.045
Ce1172_H1	C (T/F)	0.206	0.530		74.695	1.373	3.535
Ce2060_G9	C (T/F)	0.172	0.297		17.488	1.147	1.983
249M B10	C (T/F)	0.290	0.508		2.674	1.933	3.384
ZM247v1(Rev-)	C (T/F)	0.323	0.565		8.042	2.153	3.765
1394C9G1(Rev-)	C (T/F)	0.114	0.155		12.912	0.760	1.036
Ce704809221_1B3	C (T/F)	0.137	1.233		69.147	0.913	8.218
CNE19	BC	0.146	0.245		17.066	0.973	1.630
CNE20	BC	0.278	0.637		8.431	1.853	4.246
CNE21	BC	0.094	0.392		5.283	0.627	2.611
CNE17	BC	0.484	1.171		29.677	3.227	7.810
CNE52	BC	0.081	0.821		>100	0.540	5.473
CNE53	BC	0.60	3.285		60.248	3.967	21.899
CNE58	BC	0.122	0.732		51.858	0.813	4.880
MS208.A1	A	0.014	0.409		1.099	0.093	2.728
Q259.d2.17	A	1.379	1.247		48.616	9.193	8.314
Q842.d12	A	0.081	0.215		>100	0.540	1.434
0330.v4.c3	A	0.076	0.159		8.747	0.507	1.059
0260.v5.c36	A	9.0	10.948		97.906	59.953	72.986
191084 B7-19	A (T/F)	0.202	0.346		26.672	1.347	2.308
9004SS_A3_4	A (T/F)	0.251	0.484		24.314	1.673	3.227
T257-31	CRF02_AG	0.124	0.353		68.083	0.827	2.353
928-28	CRF02_AG	0.450	1.587		59.885	3.000	10.577
263-8	CRF02_AG	1.050	1.784		44.140	7.000	11.894
T278-50	CRF02_AG	3.192	5.504		43.820	21.280	36.695
T255-34	CRF02_AG	0.134	0.364		27.550	0.893	2.426
211-9	CRF02_AG	0.243	0.834		94.925	1.620	5.563
235-47	CRF02_AG	1.201	1.882		8.140	8.007	12.543
C1080.c03	CRF01_AE	0.013	0.024		82.338	0.087	0.162
R2184.c04	CRF01_AE	1.237	3.136		21.270	8.247	20.908
R1166.c01	CRF01_AE	2.876	4.213		27.921	19.173	28.090
R3265.c06	CRF01_AE	0.535	1.408		22.063	3.567	9.384
C3347.c11	CRF01_AE	0.113	1.065		13.964	0.753	7.098
C4118.c09	CRF01_AE	0.200	0.262		3.051	1.333	1.748
CNE5	CRF01_AE	0.051	0.061		3.470	0.340	0.409
BJOX009000.02.4	CRF01_AE	8.211	11.028		35.617	54.740	73.518
BJOX015000.11.5	CRF01_AE (T/F)	1.489	5.628		45.477	9.927	37.518
BJOX010000.06.2	CRF01_AE (T/F)	0.740	2.804		19.761	4.933	18.695
BJOX025000.01.1	CRF01_AE (T/F)	0.335	2.721		18.749	2.233	18.140
X1193_c1	G	0.304	1.745		16.344	2.027	11.630
X1254_c3	G	0.304	1.557		17.921	2.027	10.381
X2131_C1_B5	G	0.286	2.383		19.084	1.907	15.888
P1981_C5_3	G	2.512	5.532		71.099	16.747	36.880

X1632_S2_B10	G	0.934	5.046		96.596	6.227	33.641
231966.c02	D	0.257	0.708		14.785	1.713	4.723
3301.v1.c24	AC	0.756	2.568		24.002	5.040	17.120
6540.v4.c1	AC	0.253	0.694		55.967	1.687	4.627
6545.v4.c1	AC	0.351	1.082		25.819	2.340	7.210
Median		0.286	1.082		27.736	1.907	7.210
Mean		1.070	2.232		39.210	7.136	14.878
STD		1.943	2.967		30.891	12.953	19.777

Average decrease of potency of PG9/apl relative to PG9: 2.08 fold
Average improvement of potency for PG9/apl relative to aplaviroc: 2.64 fold

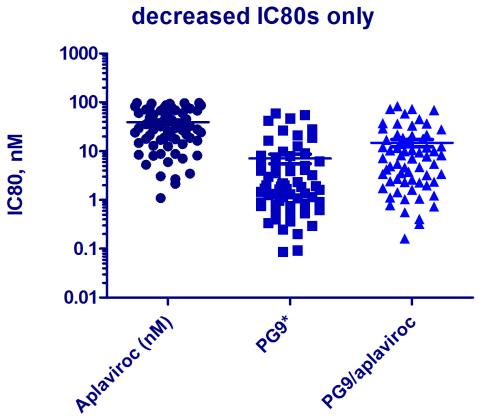


Figure 8. Graphical representation of IC80s in the pseudovirus panel where PG9-aplaviroc has decreased potency relative to PG9.

Statistical analysis for all samples with decreased PG9/aplaviroc potency relative to PG9.

The following Mean and Median IC80s were calculated by GraphPad Prism analysis:

PG9 Median IC80 = 1.91nM, Mean IC80 = 7.14nM

PG9/aplaviroc Median IC80 = 7.21nM, Mean IC80 = 14.88nM

Aplaviroc Median IC80 =27.74nM, Mean IC80 =39.21nM.

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC80 values of PG9 and PG9/aplaviroc (P value 0.0001).

Mann Whitney test has shown that in this set of samples there is statistically significant difference between Median IC80 values of Aplaviroc and PG9/aplaviroc (P value <0.0001).

6. PG9 and PG9-aplaviroc MPI full summary.

Special Note: MPIs >99.5% were rounded up by the software analysis program to 100%.

		MPI	
Virus ID	Clade*	PG9	PG9/Aplaviroc
6535.3	B	96	100
QH0692.42	B	20	78
SC422661.8	B	86	96
PVO.4	B	78	100
TRO.11	B	55	83
AC10.0.29	B	90	99
RHPA4259.7	B	63	100
THRO4156.18	B	61	93
REJO4541.67	B	96	99
TRJO4551.58	B	95	96
WITO4160.33	B	100	100
CAAN5342.A2	B	87	97
Average		77	95
WEAU_d15_410_5017	B(T/F)	64	84
1006_11_C3_1601	B(T/F)	95	100
1054_07_TC4_1499	B(T/F)	0	83
1056_10_TA11_1826	B(T/F)	83	92
1012_11_TC21_3257	B(T/F)	99	99
6240_08_TA5_4622	B(T/F)	97	98
6244_13_B5_4576	B(T/F)	27	89
62357_14_D3_4589	B(T/F)	13	97
SC05_8C11_2344	B(T/F)	93	100
Average		63	94
Du156.12	C	100	100
Du172.17	C	99	100
Du422.1	C	87	98
ZM197M.PB7	C	97	100
ZM214M.PL15	C	0	94
ZM233M.PB6	C	100	100
ZM249M.PL1	C	95	100
ZM53M.PB12	C	100	100
ZM109F.PB4	C	89	100
ZM135M.PL10a	C	69	93
CAP45.2.00.G3	C	100	100
CAP210.2.00.E8	C	98	100
HIV-001428-2.42	C	100	100
HIV-0013095-2.11	C	100	100
HIV-16055-2.3	C	100	100
HIV-16845-2.22	C	79	96
Average		88	99
Ce1086_B2	C(T/F)	0	100
Ce0393_C3	C(T/F)	100	100
Ce1176_A3	C(T/F)	100	100
Ce2010_F5	C(T/F)	23	68
Ce0682_E4	C(T/F)	100	100
Ce1172_H1	C(T/F)	100	100
Ce2060_G9	C(T/F)	100	100
Ce703010054_2A2	C(T/F)	100	100
BF1266.431a	C(T/F)	100	100
246F C1G	C(T/F)	10	100
249M B10	C(T/F)	97	100
ZM247v1(Rev-)	C(T/F)	100	100
7030102001E5(Rev-)	C(T/F)	5	92
1394C9G1(Rev-)	C(T/F)	100	100
Ce704809221_1B3	C(T/F)	100	100
Average		76	97
CNE19	BC	99	100
CNE20	BC	100	100
CNE21	BC	99	100
CNE17	BC	100	100
CNE30	BC	16	81
CNE52	BC	100	100
CNE53	BC	100	99
CNE58	BC	100	100
Average		89	98
MS208.A1	A	98	100
Q23.17	A	100	100

MPI summary continued:

Q461.e2	A	91	100
Q769.d22	A	99	100
Q259.d2.17	A	90	99
Q842.d12	A	100	100
0330.v4.c3	A	100	100
0260.v5.c36	A	94	95
Average		97	99
191955_A11	A(T/F)	100	100
191084 B7-19	A(T/F)	100	100
9004SS_A3_4	A(T/F)	100	100
Average		100	100
T257-31	CRF02_AG	100	100
928-28	CRF02_AG	100	100
263-8	CRF02_AG	99	100
T250-4	CRF02_AG	100	100
T251-18	CRF02_AG	42	70
T278-50	CRF02_AG	97	99
T255-34	CRF02_AG	97	100
211-9	CRF02_AG	99	100
235-47	CRF02_AG	97	100
Average		92	97
620345.c01	CRF01_AE	67	96
C1080.c03	CRF01_AE	100	100
R2184.c04	CRF01_AE	98	100
R1166.c01	CRF01_AE	98	98
R3265.c06	CRF01_AE	100	100
C2101.c01	CRF01_AE	99	100
C3347.c11	CRF01_AE	100	100
C4118.c09	CRF01_AE	100	100
CNE5	CRF01_AE	100	100
BJOX009000.02.4	CRF01_AE	96	97
Average		96	99
BJOX015000.11.5	CRF01_AE (T/F)	98	100
BJOX010000.06.2	CRF01_AE (T/F)	97	99
BJOX025000.01.1	CRF01_AE (T/F)	93	100
BJOX028000.10.3	CRF01_AE (T/F)	84	99
Average		93	100
X1193_c1	G	100	100
P0402_c2_11	G	95	100
X1254_c3	G	98	100
X2088_c9	G	16	91
X2131_C1_B5	G	99	100
P1981_C5_3	G	95	97
X1632_S2_B10	G	94	95
		85	98
3016.v5.c45	D	66	100
A07412M1.vrc12	D	90	99
231965.c01	D	80	94
231966.c02	D	100	100
191821_E6_1	D (T/F)	78	100
Average		83	99
3817.v2.c59	CD	97	100
6480.v4.c25	CD	13	97
6952.v1.c20	CD	35	96
6811.v7.c18	CD	3	90
89-F1_2_25	CD	88	100
Average		47	97
3301.v1.c24	AC	100	100
6041.v3.c23	AC	94	100
6540.v4.c1	AC	99	99
6545.v4.c1	AC	98	100
Average		98	100
0815.v3.c3	ACD	0	98
3103.v3.c10	ACD	58	69
Average		29	84