Neutralization Properties of Acute and Early Subtype C Human Immunodeficiency Virus Type 1 *env* Clones from Heterosexually Acquired Infections in Southern Africa

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Running head: SUBTYPE C HIV-1 GP160 REFERENCE CLONES

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ABSTRACT

The prevalence of multiple genetic subtypes and circulating recombinant forms (CRFs) of human immunodeficiency virus type 1 (HIV-1), together with the complex immune evasion strategies used by this virus, pose difficult challenges for vaccine development. In particular, these features are major obstacles for vaccines that aim to elicit a broadly effective neutralizing antibody response. Subtype C, which dominates the global HIV-1 pandemic, has been underrepresented in studies of neutralizing antibodies. Here we cloned, sequenced and characterized the biologic and neutralization properties of functional gp160 genes from eighteen acute and early heterosexually acquired subtype C HIV-1 infections in South Africa and Zambia. A general feature of these clones was their low amino acid variability (12%) in the V3 loop of gp120 compared to much greater variability (39%) immediately downstream of V3. Another distinguishing feature was their shorter and less glycosylated gp120 compared to newly transmitted subtype B viruses. Shorter gp120 sizes were most evident in the V1 and V4 loops. All clones exhibited an R5 biologic phenotype when tested as Env-pseudotyped viruses in a luciferase reporter gene assay. The overall neutralization phenotype of these pseudoviruses resembled typical primary isolates when assayed with subtype C plasma samples from South Africa. Compared to acute/early sexually acquired subtype B viruses, the subtype C viruses were more sensitive to neutralization by subtype C plasma samples (p= 0.0005) and were less sensitive to neutralization by a subtype B plasma pool (p= 0.009), suggesting a certain degree of subtype preference in the neutralizing antibody response to infection. When probed with broadly neutralizing monoclonal antibodies, the gp120 glycan-specific epitope recognized by 2G12 was absent in all of the clade C clones and most also lacked the 2F5 in gp41 but all contained the 4E10 epitope in gp41 and many also contained the IgG1b12 epitope in the CD4 binding domain of gp120. Highly conserved CD4-induced (CD4i) epitopes in the co-receptor binding domain of gp120 were weak targets for neutralization regardless of whether sub-inhibitory doses of soluble CD4 were present, suggesting that this latter region is poorly accessible on newly transmitted subtype C viruses. Collectively, these

results illustrate key genetic and antigenic properties of subtype C viruses that might impact the design and testing of candidate HIV-1 vaccines. A subset of these gp160 clones are suitable as reference reagents to facilitate standardized assessments of the neutralizing antibody response elicited by either infection or vaccination.

TABLE 1. Demographic and biologic properties of molecularly cloned, Env-pseudotyped strains of subtype C HIV-1

Env clone[a]	Panel designation	Subtype	CoR	Gender	Mode of trans.	Mo/Yr isolated	Wks after est. inf. date	Location	Plasma VL	CD4 Count	Source[b]	Accession number
Du123.6	SVPC1	C	R5	F	M-F	11/1998	12	Durban, SA	19,331	841	ccPBMC	DQ411850
Du151.2	SVPC2	C	R5	F	M-F	11/1998	6	Durban, SA	>500,000	367	ccPBMC	DQ411851
Du156.12*	SVPC3*	C	R5	F	M-F	02/1999	<4	Durban, SA	22,122	404	ccPBMC	DQ411852
Du172.17*	SVPC4*	C	R5	F	M-F	11/1998	12	Durban, SA	1,916	793	ccPBMC	DQ411853
Du422.1*	SVPC5*	C	R5	F	M-F	11/1998	6	Durban, SA	17,118	397	ccPBMC	DQ411854
ZM197M.PB7*	SVPC6*	C	R5	M	F-M	10/2002	<15	Lusaka, Zambia	n/a	n/a	ucPBMC	DQ388515
ZM214M.PL15*	SVPC7*	C	R5	M	F-M	07/2003	<13	Lusaka, Zambia	198,800	n/a	Plasma	DQ388516
ZM215F.PB8	SVPC8	C	R5	F	M-F	10/2002	<15	Lusaka, Zambia	608,560	n/a	ucPBMC	
ZM233M.PB6*	SVPC9*	C	R5	M	F-M	12/2002	<15	Lusaka, Zambia	n/a	n/a	ucPBMC	DQ388517
ZM249M.PL1*	SVPC10*	C	R5	M	F-M	08/2003	<1	Lusaka, Zambia	1,143,760	n/a	Plasma	DQ388514
ZM53M.PB12*	SVPC11*	C	R5	M	F-M	02/2000	<14	Lusaka, Zambia	26,643	n/a	ucPBMC	AY423984
ZM55F.PB28a	SVPC12	C	R5	F	M-F	08/1998	<13	Lusaka, Zambia	88,544	n/a	ucPBMC	AY423971
ZM109F.PB4*	SVPC13*	C	R5	F	M-F	03/2000	<14	Lusaka, Zambia	887,586	n/a	ucPBMC	AY424138
ZM106F.PB9	SVPC14	C	R5	F	M-F	06/1998	<18	Lusaka, Zambia	48,442	n/a	ucPBMC	AY424163
ZM135M.PL10a*	SVPC15*	C	R5	M	F-M	06/1998	<15	Lusaka, Zambia	202,999	n/a	Plasma	AY424079
CAP45.2.00.G3*	SVPC16*	C	R5	F	M-F	05/2005	5	Durban, SA	236,000	974	Plasma	DQ435682
CAP210.2.00.E8*	SVPC17*	C	R5	F	M-F	05/2005	5	Durban, SA	127,000	461	Plasma	DQ435683
CAP244.2.00.D3	SVPC18	C	R5	F	M-F	05/2005	8	Durban, SA	19,200	557	Plasma	DQ435684

[a]Clones selected as standard reference strains are marked with an asterisk.

[b]ccPBMC, co-cultured PBMC; ucPBMC, uncultured PBMC.

n/a, not available

TABLE 2. Env Amino acid sequence length comparisons between subtypes B and C.

Genomic region	Analysis groups[a]	Sequence length range	Sequence length interquartile	Sequence length median	Comparison	P-value[b]
gp120	B-ref	504-528	509-519	512	B-ref/C-ref	0.01
	C-ref	489-525	492-509	504	B-db/C-db	**8.44×10^{-15}**
	B-db	493-532	507-515	511	B-ref/B-db	0.44
	C-db	483-520	499-510	505	C-ref/C-db	0.45
V1	B-ref	23- 35	25-31	29	B-ref/C-ref	0.03
	C-ref	15- 39	17-27	23	B-db/C-db	**3.37×10^{-13}**
	B-db	17- 42	26-32	29	B-ref/B-db	0.75
	C-db	12-37	22-28	24	C-ref/C-db	0.31
V2	B-ref	39- 50	40-46	43	B-ref/C-ref	0.52
	C-ref	42- 49	42-45	45	B-db/C-db	**3.33×10^{-5}**
	B-db	36- 56	40-44	41	B-ref/B-db	0.23
	C-db	38- 52	41-45	43	C-ref/C-db	0.16
V3	B-ref	34- 35	35-35	35	B-ref/C-ref	0.36
	C-ref	35- 35	35-35	35	B-db/C-db	0.02
	B-db	33- 38	35-35	35	B-ref/B-db	0.61
	C-db	34- 37	35-35	35	C-ref/C-db	0.60
V4	B-ref	28- 35	31-33	33	B-ref/C-ref	0.005
	C-ref	20- 39	23-30	27	B-db/C-db	**1.74×10^{-12}**
	B-db	21- 40	30-33	31	B-ref/B-db	0.50
	C-db	21- 40	27-31	29	C-ref/C-db	0.06
V5	B-ref	8-18	9-10	10	B-ref/C-ref	0.57
	C-ref	8-12	9-11	10	B-db/C-db	0.13
	B-db	7-17	9-11	10	B-ref/B-db	0.26
	C-db	3-16	9-11	10	C-ref/C-db	0.83

[a]B-ref, 12 selected sequences from the subtype B panel of reference strains. B-db, 176 well-characterized subtype B database sequences. C-ref, 12 selected sequences from the C panel panel of reference strains. C-db, 122 well-characterized subtype C database sequences.

[b]Bolded p values ($p<0.002$) are significant differences after Bonferroni correction. Underlined p values ($0.002<p<0.05$) are significantly different trends after Bonferroni correction.

TABLE 3. Comparison of the number of NLG in subtype B and C gp120.

Genomic region	Analysis groups[a]	Glycan # range	Glycan # interquartile	Glycan # median	Comparison	P-value[b]
gp120	B-ref	24-29	26-28	27	B-ref/C-ref	0.01
	C-ref	23-29	24-26	24	B-db/C-db	0.004
	B-db	20-32	24-27	26	B-ref/B-db	0.02
	C-db	17-30	23-26	25	C-ref/C-db	0.86
V1	B-ref	3-6	4-5	4	B-ref/C-ref	0.06
	C-ref	2-6	3-4	4	B-db/C-db	**0.0008**
	B-db	2-8	4-5	4	B-ref/B-db	0.43
	C-db	2-7	3-5	4	C-ref/C-db	0.45
V2	B-ref	3-6	4-5	4	B-ref/C-ref	0.85
	C-ref	3-5	4-5	4	B-db/C-db	0.50
	B-db	2-8	4-5	4	B-ref/B-db	0.52
	C-db	2-7	4-5	4	C-ref/C-db	0.88
V3	B-ref	2-3	3-3	3	B-ref/C-ref	0.0036
	C-ref	1-3	1-2	2	B-db/C-db	**$< 2.2 \times 10^{-16}$**
	B-db	1-4	2-3	3	B-ref/B-db	0.4259
	C-db	0-3	2-2	2	C-ref/C-db	0.7318
V4	B-ref	3-5	4-5	5	B-ref/C-ref	0.02
	C-ref	2-6	3-4	3	B-db/C-db	0.02
	B-db	2-7	4-5	5	B-ref/B-db	0.75
	C-db	1-7	4-5	4	C-ref/C-db	0.01
V5	B-ref	0-2	2-2	2	B-ref/C-ref	0.09
	C-ref	1-2	1-2	1	B-db/C-db	0.004
	B-db	0-3	1-2	1	B-ref/B-db	0.12
	C-db	0-3	1-2	1	C-ref/C-db	0.71

[a]B-ref, 12 selected sequences from the subtype B panel of reference strains. B-db, 176 well-characterized subtype B database sequences. C-ref, 12 selected sequences from the C panel panel of reference strains. C-db, 122 well-characterized subtype C database sequences.

[b]Bolded p values ($p<0.002$) are significant differences after Bonferroni correction. Underlined p values ($0.002<p<0.05$) are significantly different trends after Bonferroni correction.

TABLE 4. Neutralization phenotypes as determined with serum samples from HIV-1-infected individuals, sCD4 and monoclonal Abs.

	ID50 in TZM-bl cells[b]																
	Reciprocal serum dilution											μg/ml					
Virus[a]	**BB8**	**BB12**	**BB28**	**BB55**	**BB70**	**BB106**	**Pool A**	**Pool B**	**Pool C**	**Pool D**	**Normal Pool**	**sCD4**	**IgG1b12**	**2G12**	**2F5**	**4E10**	**TriMab**
MN	5,107	4,573	537	2,964	43,740	203	9,013	43,740	5,550	16,952	<20	--	--	--	--	--	--
SF162.LS	15,548	5,480	2,629	20,302	43,740	552	5,597	43,264	8,066	2,438	<20	--	--	--	--	--	--
Du123.6	207	352	165	84	147	198	306	182	18,845	220	90	0.3	0.2	>50	>50	0.1	1.6
Du151.2	196	1,555	2,529	818	241	487	347	518	527	123	20	3.0	1.4	>50	>50	0.8	13.5
Du156.12*	369	426	238	336	406	258	810	231	2,368	228	52	13.4	0.8	>50	>50	0.2	1.2
Du172.17*	429	884	499	196	549	550	462	315	629	562	91	1.7	1.0	>50	>50	0.3	2.6
Du422.1*	134	354	193	91	51	63	335	114	2,138	65	90	9.1	0.2	>50	>50	0.7	0.8
ZM197M.PB7*	79	103	76	117	348	68	163	185	332	176	<20	3.9	19.9	>50	12.3	0.5	24.8
ZM214M.PL15*	259	366	104	90	206	96	127	<20	162	64	<20	8.0	3.0	>50	>50	4.0	9.7
ZM215F.PB8	231	694	122	384	90	<20	165	51	706	108	32	14.0	>50	>50	>50	0.4	>25
ZM233M.PB6*	295	153	214	413	2,899	80	127	108	640	218	<20	2.9	>50	>50	>50	1.2	>25
ZM249M.PL1*	160	105	287	158	112	117	193	63	383	156	33	9.7	3.2	>50	>50	2.1	7.9
ZM53M.PB12*	76	73	411	86	<20	98	57	91	566	65	<20	8.3	25.9	>50	>50	7.0	>25
ZM55F.PB28a	96	206	106	169	47	110	99	56	250	59	27	24.0	>50	>50	33.6	8.0	>25
ZM109F.PB4*	160	188	89	160	238	61	185	82	365	119	<20	0.2	>50	>50	>50	0.6	>25
ZM106F.PB9	249	378	287	262	68	71	101	76	433	<20	<20	23.0	>50	>50	>50	7.2	>25
ZM135M.PL10a*	138	179	93	190	50	177	108	57	202	109	29	6.1	>50	>50	>50	0.6	>25
CAP45.2.00.G3*	61	437	351	44	170	52	142	47	453	60	<20	26.0	0.7	>50	>50	2.6	1.5
CAP210.2.00.E8*	111	75	82	113	272	75	101	29	293	51	<20	3.4	20.4	>50	>50	1.2	20.8
CAP244.2.00.D3	53	47	32	51	91	30	38	27	94	40	<20	8.9	>50	>50	>50	1.9	>25
GMT[c]	151	227	177	151	142	97	158	79	514	98		5.3				1.1	

[a]Clones selected as standard reference strains are marked with an asterisk.

[b]Values are the dilution or concentration at which RLU were reduced 50% compared to virus control wells. BB8, BB12, BB28, BB70 and BB106 are plasma samples from individuals infected with subtype C HIV-1.

[c]Geometric mean titer against the 18 pseudoviruses containing cloned primary isolate Env (excludes MN and SF162.LS). Serum/plasma titers <20 were assigned a value of 10 for calculations. Because multiple ID50 values were >50, GMT was not determined for IgG1b12, 2G12, 2F5 and TriMab.

TABLE 5. Neutralization with CD4i monoclonal Abs is the presence and absence of sCD4.

	ID50 in TZM-bl cells[b]								
Env clone[a]	**17b**	**23e**	**31H**	**21c**	**E51**	**48d**	**112d**	**412d**	**ED10**
NL-ADRrs	0.002	0.002	0.002	0.002	<0.0001	0.2	6.1	0.002	2.9
SF162.LS	0.9 / 2.3	1.3 / 3.7	1.0 / 2.9	1.1 / 3.5	0.1 / 0.6	4.6 / 12.6	- / -	0.2 / 1.4	3.0 / 5.9
Du156.12*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Du172.17*	- / -	- / -	- / -	- / -	- / 4.7	- / -	- / -	- / -	- / -
Du422.1*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
ZM197M.PB7*	- / -	- / -	- / -	- / -	19.5 / 21.6	- / -	- / -	16.7 / -	- / -
ZM214M.PL15*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
ZM233M.PB6*	- / -	- / -	- / -	- / -	6.5 / 10.2	- / -	- / -	- / -	- / -
ZM249M.PL1*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
ZM53M.PB12*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
ZM109F.PB4*	- / -	- / -	- / -	- / -	9.9 / 16.8	- / -	- / -	19.3 / 19.1	- / -
ZM135M.PL10a*	17.0 / -	5.9 / -	- / -	- / -	17.6 / -	- / -	- / -	- / 20.7	- / -
CAP45.2.00.G3*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
CAP210.2.00.E8*	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -

[a]Clones selected as standard reference strains are marked with an asterisk.

[b]Values are the monoclonal Ab concentration at which RLU were reduced 50% compared to virus control wells. Neutralization was measured in the absence / presence of sCD4. sCD4 was present in all wells (including cell control and virus control wells) at a concentration equal to the ID50 for each virus. Dashed lines signify no neutralization at the highest concentration of monoclonal Ab tested (25 μg/ml). NL-ADArs was assayed in the absence of sCD4 only.

FIG 1. **Phylogenetic relationships of subtype C reference Env clones**. The newly characterized sequences are indicated by boxes and the 12 *env* clones that are recommended as standard reference reagents are highlighted by shaded boxes. Horizontal branch lengths are drawn to scale (the scale bar represents 0.01 nucleotide substitution per site), but vertical separation is for clarity only. Values at nodes indicate the percentage of bootstraps in which the cluster to the right was found; only values of 80% or greater are shown. The phylogenetic tree was rooted with subtype D *env* sequences (NDK, Z2Z3 and 94UG114).

FIG 2. **Alignment of deduced amino acid sequences from acute/early subtype C HIV-1 *env* genes**. Nucleotide sequences of newly derived *env* genes were translated, aligned, and compared with a consensus sequence generated by MASE. Numbering of amino acid residues begins with the first residue of gp120 and does not include the signal peptide. Dashes denote sequence identity, while dots represent gaps introduced to optimize alignments. Small letters in the consensus sequence indicate sites at which fewer than 50% of the viruses share the same amino acid residue. Triangles above the consensus sequence denote cysteine residues (solid triangles indicated sequence identity, while open triangles indicate sequence variation). V1, V2, V3, V4, and V5 regions designate hypervariable HIV-1 gp120 domains as previously described. The signal peptide and Env precursor cleavage sites are indicated; msd denotes the membrane-spanning domain in gp41; asterisks mark in-frame stop codons. Open circles highlight altered cysteine residues. Potential N-linked glycosylation sites (NXYX motif, where X is any amino acid other than proline and Y is either serine or threonine) are bolded and underlined.

FIG. 3. **Neutralization-sensitivity of acute/early subtype C and subtype B HIV-1 Env-pseudotyped viruses as determined with plasma samples from HIV-1-infected individuals**. Bar height represents the geometric mean titer (GMT) of neutralizing Abs against the indicated Env-pseudotyped viruses. A. GMT for all HIV-1-positive plasma samples shown in Table 4 (BB8, BB12, BB28, BB55, BB70, BB106, Pool A, Pool B, Pool C and Pool D). Clones selected as standard subtype C reference strains are marked with an asterisk. B. GMT of neutralizing Abs for the individual subtype C plasma samples BB8, BB12, BB28, BB55, BB70,

BB106 assayed against the twelve subtype C reference strains. C. GMT of neutralizing Ab in the individual subtype C plasma samples BB8, BB12, BB28, BB55, BB70, BB106 assayed against twelve subtype B reference strains.

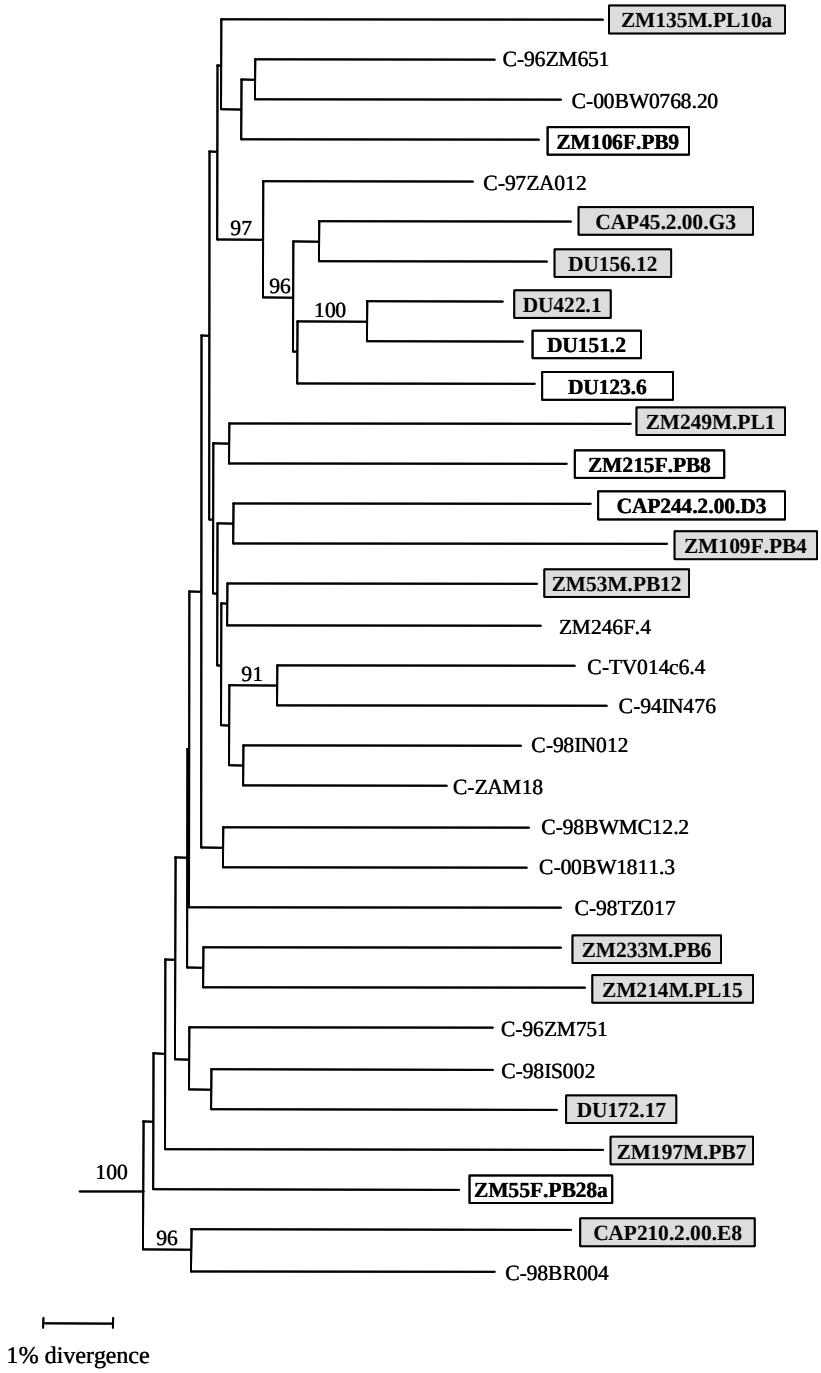
ZM135M.PL10a
C-96ZM651
C-00BW0768.20
ZM106F.PB9
C-97ZA012
97
CAP45.2.00.G3
DU156.12
96
100
DU422.1
DU151.2
DU123.6
ZM249M.PL1
ZM215F.PB8
CAP244.2.00.D3
ZM109F.PB4
ZM53M.PB12
ZM246F.4
91
C-TV014c6.4
C-94IN476
C-98IN012
C-ZAM18
C-98BWMC12.2
C-00BW1811.3
C-98TZ017
ZM233M.PB6
ZM214M.PL15
C-96ZM751
C-98IS002
DU172.17
ZM197M.PB7
100
ZM55F.PB28a
96
CAP210.2.00.E8
C-98BR004
1% divergence

FIGURE 1

```
                                signal peptide  ↔  gp120
Consensus          MRVrGILRNWQQWWIWGILGFWMLMICnVV.GNL..WVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEmVLEN
DU123.6            ---K--Q---P------------II--R--.---..-----------T----------------R--------------------I--G-
DU151.2            ---RE----Y-------T---------N--.---..----------------------------D---------------------I---
DU156.12*          ---R--P---P---T--------IIM-K-A.--S..-----------T-----------------------------------IF-K-
DU172.17*          ---M----SY-----------------N-W.---..---------------------------HKE----I---------------I--K-
DU422.1*           ---R--P---P------------II--R--.---DL---------------------------D---------------------I---
ZM197M.PB7*        ---M-----------------------NM..EQ-..---------------A--------------------------------IP-G-
ZM214M.PL15*       ---R-M---Ⓒ-----------------NG-.---..-----------R-------------------------------------L---
ZM215F.PB8         ---M-----Ⓒ-------------..--N--.---..---------------A----------Q-T----I---------------MI--
ZM233M.PB6*        ---R--M----------S------I--N-M.-S-..-----------R----------------T-A-S----------------M---
ZM249M.PL1*        ---M-----Ⓒ-P----S--------N-S..---..-----------R----------------------V-------------MN--
ZM53M.PB12*        ---RE-P--Y-----------------S--.---..-----------R----------------R------------------M---
ZM55F.PB28a        ---K-MK-----------------..ⓎSGM.-S-..----------------------------R--------------------M---
ZM109F.PB4*        ---K-----Ⓒ-----------------N--.---..---------------------------S--R----------------D---L-MA-
ZM106F.PB9         -K-RE-----R----------------N--V---..----------------------------R----------------S---M---
ZM135M.PL10a*      ---R--Q----------S-----F---SG-.---..-----------R-------------------------------------L--G-
CAP45.2.00.G3*     ---R------P-----S-------I--R-M.---..---------------A--------R-----------------------IY-G-
CAP210.2.00.E8*    ---M--Q------G--------L----SGM.---..-------------------------G-DT--------------------I---
CAP244.2.00.D3     ---K-M---Y-------------I-L-N--GQ--..----------------------N--------------------------M--G-
                                                                                                          90

                                                                                  ┌──────────────V1──────────────┐
Consensus          VTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTPLCVTLNCt....................naTyNnsm......gEiKNCSFNiTT
DU123.6            --------------------I------------------------T DVK.......VNATSNGTTTYNNSIDSM.....NG-I-----I--
DU151.2            --------------------------------------------T...................NAPAY-NSM.....HG-M-----T--
DU156.12*          ------------------------------------------VT............YNNSMNSSA-Y-NSM.....NG-I-----T--
DU172.17 *         ------------------------------------------SDVKIKG........TNATYNNA-Y-NNNT....ISDM-----T--
DU422.1*           -----------------------------------------KNVNIS..........ANANATA-L-SSM.....NG-I-----T--
ZM197M.PB7*        ----------A-----------------------------SDAT...SNTTKNATNTNTTSTDNR-ATSNDTEMKG-I-D-T--I--
ZM214M.PL15*       ------------N---------------------------SNVN.........INETSIDFNV-S-ISM.....KE-M-----KVNS
ZM215F.PB8         ------------------V-----------I---------T...................NA-Y-GST.....IEDVR-----A--
ZM233M.PB6*        ------------------V--I-----------------D-S...................TYNN-H-ISK........-M-I---M--
ZM249M.PL1*        -----------------------------------------NNV..............NVTHNS-Y-NTE.....GEQI-----I--
ZM53M.PB12*        --------------Q------------------------S.....................KL-NAT.....DG-M-----A--
ZM55F.PB28a        -----------------------E---------------T.....................FITNTT........-I---T--M--
ZM109F.PB4*        ------------------------------------------T...................SPAAH-ESE......TRV-H---I--
ZM106F.PB9         -------------------------------------K-VNVNAT...........SKSNASAT-DGS......G-M---T--I--
ZM135M.PL10a*      -------E-----------------------------E-KNAT..............RSNQT-YYDNM.....DK-I-----V--
CAP45.2.00.G3*     -------------------------------------R-T...................NA-I-GSL.....TE-V-----I--
CAP210.2.00.E8*    ---------------Q------------------------S.....................DA-Y-NGT...NSTDTM-I---A--
CAP244.2.00.D3     ------------------------E---------------I-TNVNHSSTNVSHSSTNVSQSSNSSQDNTTIDESMRE-I---Y-S--
                                                                                                          180

                   ──────────────── V2 ────────────────┐
Consensus          ELRDKKQKvYALFYKLDIVPLngnsnss.......EYRLINCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNNKTFNGTGPCNNVST
DU123.6            -I------V-----RP-V---NENSSS.........-I-------T-------------------------------------H----
DU151.2            -I--R---A------P-V---NRREENNGTG......-I-----S----------T-------------------------------
DU156.12*          --------V-----RT-V---NNNNNNS.........-I-------------------------------TD-K----S-------
DU172.17*          -IT---K-E--------V-A-DGKETNSTNSS...-------------AV-------------------------------------
DU422.1*           --------V------P-V---NGGEHNETG......-I-----S-------------------------------------------
ZM197M.PB7*        -V--R-T-QR-------V---EEEKNSSSKNSSYK-----S-------------------------------------------H----
ZM214M.PL15*       -----NRREH---------Q-NDEGNDSYS.....-------------K--------E-------------E-----S---------
ZM215F.PB8         -VKN---EVN---NL-----IKSSI...........---T-------A--------T-----------------------K-------
ZM233M.PB6*        ------R-VNV------L---TNSSNTTN.......----S-------------------------------------------
ZM249M.PL1*        --------V---------L--NGNNDSN.......-------------A-------------------------------K-------
ZM53M.PB12*        ------KQV------------DGRNNSS.......-------------------------------------------H----
ZM55F.PB28a        ----I--QGR---DT------KPPNNSSNYS....-----S-------------------------------------L-------
ZM109F.PB4*        DVK-R---VN-T--D------SSSDNSSNSS....L----S-------------------------------------S-K---S----
ZM106F.PB9         -I----RNES-----------TNDNNSG.....-------------AM-------------------------------Y----
ZM135M.PL10a*      --T---KNMR----RA--E--DGNSNESINSSEGDK-I-------------A------T-----------------I---K----
CAP45.2.00.G3*     --------A-----RP-V---NKNSPSGNSS.....-I-------------------------------------------
CAP210.2.00.E8*    ------K-E-----R------KNESESQNFS.....-I-------------A-------------------------------
CAP244.2.00.D3     --W-----E-----------INGNAS.........-------------K------T-E-----------------Q-------T----
                                                                                                          270
```

```
                                                                                                  V3
Consensus        VQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTNNvKTIIVHLNESVEIvCTRPNNNTRKSIRIGPGQTFYATGdIIGDIRQAHCNISk
DU123.6          ------------------------------------------A--------I--V-------------V---ND------------K
DU151.2          -------N----------------------------I--------K----V-------R--------------E---N--E----K
DU156.12*        ------------------------K-----D-I-----Q--Q-IG-N-----------V------------D------------R
DU172.17*        ------------------------------VV--F------A-I----------N----S----------V--------F---D------------R
DU422.1*         ------------------------V-------I--------K----K----------V------------E------E-----R
ZM197M.PB7*      ------------------------------D-T--------------E-V-----------V--------F---E----------DL-K
ZM214M.PL15*     ------------------------K--M-------A-----Q-T-A-N-T-M--G----R-V------------E------------K
ZM215F.PB8       ------------------------------D--------V--------K-P---V----G----------V------------A------K-----K
ZM233M.PB6*      ------------------------------F----D-V-I---Q--TIN-T-----------------S-----E-V-N--E-----A
ZM249M.PL1*      ------------------------K--------I-D-V-I-------------N-----------------------E---K--E-----K
ZM53M.PB12*      ----------I----------T---D-------------A----------I--E----G-------------A-F--TN--------Y-I-NK
ZM55F.PB28a      ------------------------------------------V----------P-Y-V-----------M------------D------------I
ZM109F.PB4*      -------R----------------------V-----D-A--------K----E-I--G----------L----------DV-----K-Y-K-NG
ZM106F.PB9       ------------------------G----------D-V--------IH-T-----------------------E------K-Y-----E
ZM135M.PL10a*    ------------------------S--G-----K----D-T--------A-V-----------------------EV------------G
CAP45.2.00.G3*   ------------------------D---K-------I--------K----V-R-----------A----ND------------NN
CAP210.2.00.E8*  ------------------------VV-----IS--V----------N-T-I--G----R--------A---M-D---N--E-----E
CAP244.2.00.D3   ------------------------K----S-------A-I-----KDP-R-V-----------------D--------Y---DK
                                                                                                    360

                                                                                V4
Consensus        skWNtTLerVkkKLkEHFPNKTI.KFEPSSGGDLEITTHSFNCRGEFFYCNTSkLF................n?tsnnttnaTITLPCRI
DU123.6          TK--T--EK-KE--K----S-A-.T-Q-H------V--------------D-TK--................NESNLNTTNTT-L------
DU151.2          SS-TS--EQ-KK--K--Y.----.E-N-P------V--------------T-K--..................SNNSDSNNE-------K-
DU156.12*        NQ--E--EQ-KK--G---H-Q-KI----P-----------------------AD--............TNATKLVNDTENKAV--I----
DU172.17*        KK--T--QR-KE--K-K-------.Q-A-----------------------Y--D--...............NSTYMSNNTGGAN---Q---
DU422.1*         ET--S--IQ-KE--R--Y.----.-----------V--------------D-TK--............NETKLFNESEYVDNK--I-----
ZM197M.PB7*      SN-TT--KRIEK--K---N-A--.---S-A------------------------G--........NSSLLNDTDGTSNSTSNA-------
ZM214M.PL15*     DK--QI-QN-RA--G---HD---.------------------G-------T-N--........SRTYTNGSNSNVNITSA--------
ZM215F.PB8       KA--D--HK-EG--R-LY.--S-.---------------------------T-K--................HEGSRKNDND-------
ZM233M.PB6*      SK--K--ER-RT--K-------.E------------------G-------G--................NSAINGTLTSNV------
ZM249M.PL1*      EK--K--LR-AK--R----G-A-.---------------------------T--K--........NSTYNPNDTESNSNNSNE-L--T-K-
ZM53M.PB12*      AN-TN--HR-SK--E-------.N-NS-----------------G-------S--............NGTYNDTDIYNSTD-I-L---
ZM55F.PB28a      EK--T--EK-KER-KK-----I-.-----------------------------AN--...........NETFMNQTDANQTNA---Q---
ZM109F.PB4*      SE--E--TK-SE--K-Y-.----.R-AQH------V----------------E--...................NSNATESN--------
ZM106F.PB9       EK--KA-QE-GK--K-------.--A-----------------------K--...........NSTYMHNATSRNATNA--------
ZM135M.PL10a*    EQ--R--ERIKD--T-Y--D-I-.--NH-----------T----------I--..................TENENSSDN--------
CAP45.2.00.G3*   ST--R--EQIKK--R---L-R--.E---P------V-------G-------T-R--................KWSSNVTND---I----
CAP210.2.00.E8*  KA--E--KK-VE--VKY-----.E-A-PV-------------G-------T-K--NSTHNSTDSTVNSTDSTAETGNSTNTN--------
CAP244.2.00.D3   GA--K--QQ-GK--Q----G---.--NS-----------------------K--...........NNTYYNGTGNANSTHEN--------
                                                                                                    450

                                                  V5
Consensus        KQIINMWQeVGRAMYAPPIaGNITCnSNITGLLLTRDGG....nknNtTEtFRPGGGDMKDNWRSELYKYKVVEIKPLGIAPTKAKRRVV
DU123.6          ---V----G---------VE----N-S------V----...NTSNSTP-I------N--------------------V----------
DU151.2          --------K----------E----K-------------.....KNTTN-I------N----------------E---V----S-----
DU156.12*        --------G----------E----N-------------.GNVTEI-R--I------N------N--------------V---G---K--
DU172.17*        ----R---G--Q-------A----K-------------....KEK-D--T--------R---------------------D-------
DU422.1*         --------E----------E----K----------W---.....ENSTEGV------N---------------------V----S--K--
ZM197M.PB7*      --------E-------S--A-I---K-------------....NKSAGI-T------N-----------------------S------
ZM214M.PL15*     --------E----------A----I-------------..NGNDT-D--T---A----R-----------------------------
ZM215F.PB8       --F-----E----------A----N-S---I--V----.....TN-TN-T--------R---------------Q--------------
ZM233M.PB6*      --------E----------A----K-------------...ENSSST--T---T-----N----------------------E------
ZM249M.PL1*      --------G----------E-S---N-T-----------....SKN-TE-I------N--------------------V---E------
ZM53M.PB12*      --------E----------E----S-------------....LTNESK-T--------R---------------------------
ZM55F.PB28a      --------G----------P-R---N-S----I-------..ENTTD-G--I--------R----------------------E------
ZM109F.PB4*      --------G----------R-E-K-T-------------...NNNNSTE-I---E--N-R----------------------E------
ZM106F.PB9       R-------E----------A----V-------V----...NGDT-D--T---------N------------------V---E------
ZM135M.PL10a*    --FV----E----------A----N-S-----------..LNNKE-G--T---Q----R---------------R---V----------
CAP45.2.00.G3*   --F-----GA---------E----N-S-----------...KTDR-D--I------N------N--------------V---E-R----
CAP210.2.00.E8*  R-------E---------SK----I-------------.ENKTEN-D--I----------------------------V---R------
CAP244.2.00.D3   ----R---K--Q-------A----T-------V----..EANGT-NI-T--------R---------------Q--------------
                                                                                                    540
```

```
                 gp120 ←V→ gp41
Consensus        EREKRAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYLKDQQLLGLW
DU123.6          ------------LF------------------------------------------------------------------------
DU151.2          --------L---L-------------------------------------------------------------T------------------
DU156.12*        K-------L---LF------------------A-------------------------------------------------------
DU172.17*        ----------------------------M-----------------------------------------------T----------------I-
DU422.1*         G-------L---L-------------------------------------------------L------------T------------------
ZM197M.PB7*      ------A-----I---------------VM-----------------------------------------------T------------------
ZM214M.PL15*     G-------------------------------------------------N-----------L------------------------------
ZM215F.PB8       --------L-----------------------A-------------------------------------------------------Q------I-
ZM233M.PB6*      ----------------------------M--------------------K-------------------------------------------
ZM249M.PL1*      --------L-------------------------------------N-----------------------------------------------I-
ZM53M.PB12*      --------L--M----------------------------------N-----------------------------------------------
ZM55F.PB28a      ------------------------------------------------------------------------------------------------I-
ZM109F.PB4*      Q-------------------------------------------------------------L----------------------Q------I-
ZM106F.PB9       ------------L-------------------A----V--------------------L------------T----L-------------
ZM135M.PL10a*    -K----------------V-------------A----V-------------------------------------------------------I-
CAP45.2.00.G3*   ------------L-------------------------------------------------------------T------------------
CAP210.2.00.E8*  ------------------------------------------------------------------------T----------------I-
CAP244.2.00.D3   -----------M----------------MA----S-----------------------------------R---V-L----Q------I-
                                                                                                          630

                 ▼     ▼                                                                                 ┌──
Consensus        GCSGKLICTTAVPWNSSWSNKSqtDIWDNMTWMQWDREISNYTnTIYrLLEdSQNQQEKNEKDLLALDSWkNLWNWFdITNWLWYIKIFI
DU123.6          --------P-T---------QT-------------------G---K---E------------------K---S--D------------
DU151.2          -----I--------------QE-------------------G---R---D------------------K------N-----------
DU156.12*        ----------N---------QT---N-T-----E-------D---R---D------E---------R-Q------D------------
DU172.17*        ---------------A-------YEE--G-----------N---N---S---E------------------ES--S--N--------R---
DU422.1*         --------A-----------LG-------------------N--FR---D------------------K------D------------
ZM197M.PB7*      ---------------T-------KDE--------------D---QV--Q---V---------N------K-NS--S--D--K---------
ZM214M.PL15*     ----------T-S---------VD---Q-----------N---EI--R---V---------E------K-D------D-SK---------
ZM215F.PB8       ---------------------R-QA---E-------EK------N---W---D--I---------------K------NM----------
ZM233M.PB6*      ----------N---A-------KN----------------H-D---R---D------------------K------S--K---------
ZM249M.PL1       ----------S---T-------KAE-----------K------Q---N---E--S----------E----N------D-SK---------
ZM53M.PB12*      ------V-------------QE---N-T------K-V---K---K---K------E-----------N------D-S----------
ZM55F.PB28a      ----------N---------LG---------E--------NI-FG---D------R---------K-N------N--Q---------
ZM109F.PB4*      ---------------------KEE--G--------K-V---F---Q---E--Y---Q---E----NK-ND--S--N-----------
ZM106F.PB9       ----R---------------LT-----------K-V---N---R---D--S---------------K---T--D-S----------
ZM135M.PL10a*    --------------ED---RTQEE--N--------K------D---K--TE--S--D------------K------D--Q-----R---
CAP45.2.00.G3*   ----------N---------QT--------I---------SN---K---G------Q-----------N------N-----------
CAP210.2.00.E8*  ----------N---------YG---------------N---N---R---D---------Q------K-QS--S--S-SS---------
CAP244.2.00.D3   ----------N---------R-.A-----L-----EK--N---D---Q---E--I------Q-----N--DS--S--S--K---------
                                                                                                          720

                 ________ msd ________┐
                                                                                                ▼
Consensus        MIVGGLIGLRIIFAVLSIVNRVRQGYSPLSFQTLTPNPRGPDRLGRIEEEGGEQDKDRSIRLVNGFLALAWDDLRSLCLFSYHRLRDFIL
DU123.6          -------------G-----K--------------------L-----------------------------------------------
DU151.2          -------------G--A--K----------------S-------------------N------S-----------------------L--
DU156.12*        -------------G-----K---E------------T---L-------------------------------------------Q------
DU172.17*        I-----------------------------------S--E---------------RA--V-----------E---------------L--
DU422.1*         -------------G--A--K--------------I----------------------------S-------------------Q------
ZM197M.PB7*      -----------------V----------------I--------P-G----------N--T---S-----V--------------------
ZM214M.PL15*     ---------------------------------------EL--------------RS--------------------------H---L--
ZM215F.PB8       ----------------------------------I---------E---------------------------------I---------M-
ZM233M.PB6*      ---------------------------------------------G----------NK-R---T---PVV-------------L------
ZM249M.PL1       --------------------------------I----------------------R---V--A--------E---N----F---------
ZM53M.PB12*      ----------------------------------Q----L--------------R---V---------F--------------------
ZM55F.PB28a      ----------------------------------F----EL---------------G------S---T-----V-------------©--
ZM109F.PB4*      ---------------------------------------G---------G-------N-----------I----W---R----L------
ZM106F.PB9       -V-------------I--------------L----QS-G----------------R-----------T----------------------
ZM135M.PL10a*    ------------------------------L---I----EL---------------S------H-------------------------
CAP45.2.00.G3*   --I---------LG-----K--------------------L------------------------------E------------------
CAP210.2.00.E8*  -V----------------------------L---P----EL----G---------RG--V---S---P------------©------LL-
CAP244.2.00.D3   --I-----------------------------------------E----G-------------S-----I--------------------
                                                                                                          810
```

```
                                                                                                                  ▽
Consensus        IAARAVELLGRSSLrGLQRGWEALKYLGSLVQYWGLELKKSAISLLDTIAIAVAEGTDRIIEliQRIcRAIrNIPRRIRQGFEAALL*
DU123.6          V-------------R-------------N----G-----RR----F--------------L-VIL--I---R---T-----------*
DU151.2          VVT-----------R-------------N----G-----R---K-F--------------L-VI---©---RH--I-----------*
DU156.12*        --------------R---K---------N-I--------RR--N---IS------------DIVL-TG---R----------G-T--*
DU172.17*        -----AA-------W---K-------------------------F-A---T---------NIV---S--FY------------T-Q*
DU422.1*         T----A--------R-------V-----N----------R---N-F----------------VI---©---RY--T-----------*
ZM197M.PB7*      --------------E---W---T----RN----------Q---N-------Q----------LI---F---©---T----------Q*
ZM214M.PL15*     ----T-S----R.......----------G------R------------V--T-------V--IA--FG-G-©-------------Q*
ZM215F.PB8       VT------------R------T-------------Q------------V-------------LV---©-G-LH--T-----L----Q*
ZM233M.PB6*      -V--T------R.......----------G------------T---------V----------VL---G---Y-----------T---*
ZM249M.PL1       ----T----RQI-FK-------------------SQ---E---N--N---------------VV--GF---L-V-T-----L-R---*
ZM53M.PB12*      --T-V---------K---------R----R--------------F----------------LI--SW---R-----------T---*
ZM55F.PB28a      -V--V---------R---Q---T------------Q-------------------------II---©---R--------------Q*
ZM109F.PB4*      -V------------K-------------N-M--------R---N----T-V-----------LA-G-Y---©-------------Q*
ZM106F.PB9       ----V-----H---R---K---------------©----R-------S--M-----------LV---©-G-YH-------------Q*
ZM135M.PL10a*    VV--------HA.......---T--------------------N-F----------------LV---©---G-----V--------Q*
CAP45.2.00.G3*   --V-------S---R---------------L------------N----V-------------LI---©---R---------------*
CAP210.2.00.E8*  -TT------A--I-K-------I------------Q-------N----T------AA---L-LL---W-G-©-V-T----------Q*
CAP244.2.00.D3   --------------K-------------N-------------V-------------------LV-A-G---R---T-----------*
```

FIGURE 2

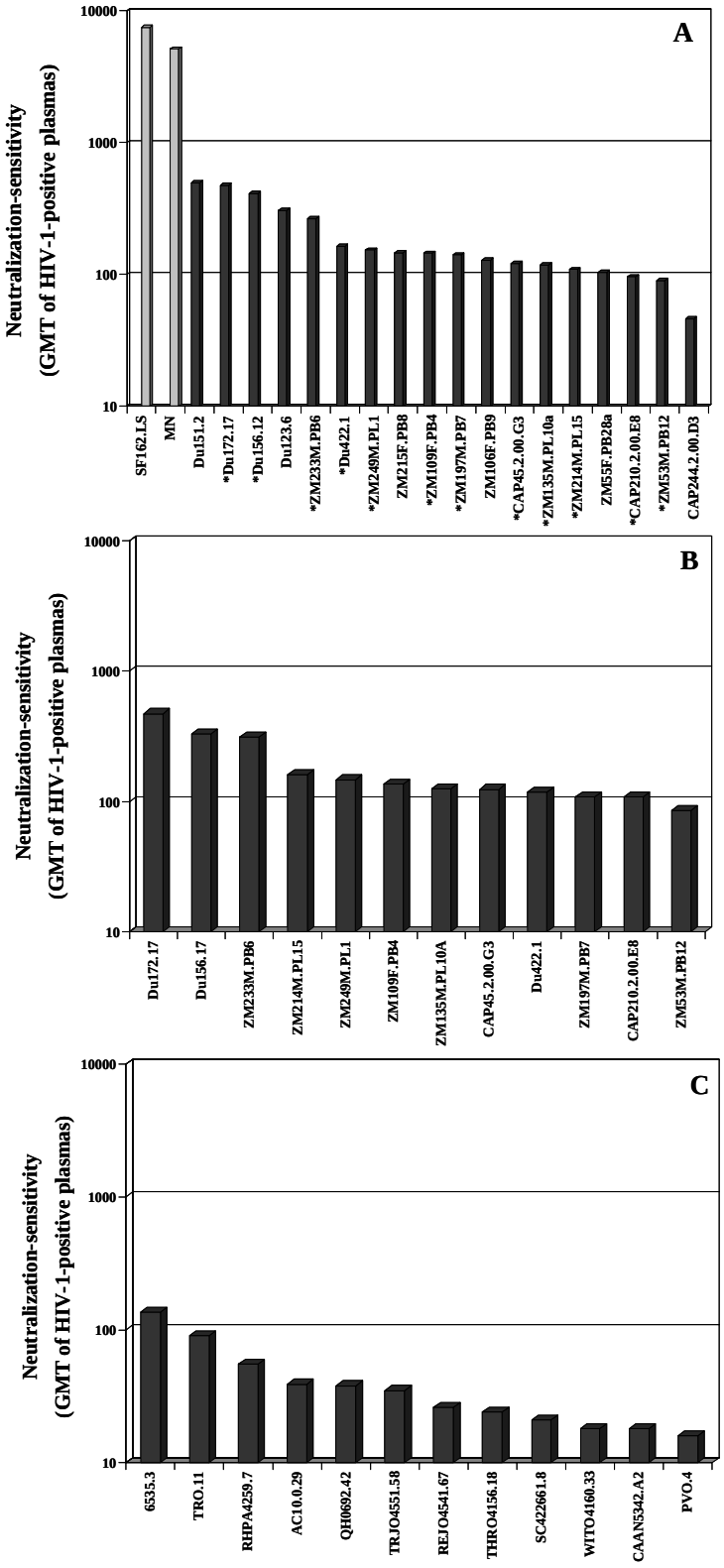
A
Neutralization-sensitivity
(GMT of HIV-1-positive plasmas)
10000
1000
100
10
SF162.LS
MN
Du151.2
*Du172.17
*Du156.12
Du123.6
*ZM233M.PB6
*Du422.1
*ZM249M.PL1
ZM215F.PB8
*ZM109F.PB4
*ZM197M.PB7
ZM106F.PB9
*CAP45.2.00.G3
*ZM135M.PL10a
*ZM214M.PL15
ZM55F.PB28a
*CAP210.2.00.E8
*ZM53M.PB12
CAP244.2.00.D3
B
Neutralization-sensitivity
(GMT of HIV-1-positive plasmas)
10000
1000
100
10
Du172.17
Du156.17
ZM233M.PB6
ZM214M.PL15
ZM249M.PL1
ZM109F.PB4
ZM135M.PL10A
CAP45.2.00.G3
Du422.1
ZM197M.PB7
CAP210.2.00.E8
ZM53M.PB12
C
Neutralization-sensitivity
(GMT of HIV-1-positive plasmas)
10000
1000
100
10
6535.3
TRO.11
RHPA4259.7
AC10.0.29
QH0692.42
TRJO4551.58
REJO4541.67
THRO4156.18
SC422661.8
WITO4160.33
CAAN5342.A2
PVO.4

FIGURE 3