

Poster 764-W

HIV-1 Group M and Group O Dual Infections in Rural Northwest Cameroon

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ABSTRACT

Background and Methods: HIV-1 has high genetic diversity and is classified into group M (subtypes A-K, CRFs, mosaics), group N, and group O. HIV diversity was examined in a rural population in the Northwest province of Cameroon. Patients attending local hospitals (1998-2000) were screened for HIV using Determine HIV-1/2, a rapid test for antibodies to HIV-1 group M and O, and HIV-2. Research assays were used to serotype the HIV infections: MO2N assay (gp41 based), *env* V3 peptide EIA (PEIA), and *env* IDR PEIA. RT-PCR followed by sequencing was used to genotype HIV strains.

Results: Determine HIV-1/2 positive specimens (N=3114) were collected and classified by serology as: 11 group O, 3 HIV-1 group M and O dual infections, 1 HIV-2, 2 HIV-1 group M and HIV-2 dual infections, and the remaining as HIV-1 group M. RT-PCR confirmed the presence of group O virus in 10 of the 11 group O seroreactive specimens. Using group M or O specific PCR for *gag*, *pol*, and *env*, sequences for both group M and group O were amplified from the 3 dual M/O seroreactive specimens. For all 3 specimens, group M PCR amplified sequences that cluster with group M and group O PCR amplified sequences that cluster with group O. Since both group M and O sequences were obtained from each specimen, the specimens are infected with at least two HIV strains. The vast majority (83%) of the group M specimens, that were evaluated (N=167), are subtype A (most cluster with CRF02_AG). Subtypes D (2.4%), F2 (2.4%), G (3.6%), and mosaics (8.4%) were also found.

Conclusions: The HIV diversity in the rural population of northwest Cameroon is high with group M subtypes A, D, F2, G, and mosaics, and diverse group O isolates found. Considering the low prevalence of group O infections in northwest Cameroon (0.45%), it is surprising that 3 of 13 confirmed group O infected patients are also infected with a group M virus. Given that most serological screening tools do not easily discriminate between cross-reactivity and dual infections, group M and O dually infected individuals may be more common than expected. Recombination between group M and O may lead to new viral strains with unknown properties.

Serological Screening Algorithm

Field Sites

Screen with rapid assay

Determine™ HIV-1/2



Collect HIV positive specimens



Abbott Laboratories

Retest for HIV Infection

HIVAB HIV-1/HIV-2 (rDNA) EIA (3A77)

+

Rapid MO2N (Research Use Only) Test



HIV positive, grayzone, discordant specimens



V3 Peptide EIA



IDR Peptide EIA



Serological Classification

Serological Classification









HIV-1 Group M	3097	(99.5%)
HIV-1 Group O	11	(0.35%)
HIV-2	1	(0.03%)
HIV-1 Group M/O Dual	3	(0.10%)
HIV-1/HIV-2 Dual	2	(0.06%)
TOTAL	3114	

Figure 2: Schematic of Peptide Immunoassays









The PEIAs are performed in a 96 well microtiter plate with each peptide coated across a row and specimen added down a column.

Direct Binding PEIAs

V3 PEIA

-  No peptide
-  Group M
-  Group O ANT70
-  SIVcpzANT
-  SIVcpzGAB
-  Group N
-  All V3
-  All IDR

IDR PEIA

-  No peptide
-  Group M
-  Group O ANT70
-  Group O MVP5180
-  SIVcpzANT
-  SIVcpzGAB
-  Group N
-  All IDR

Peptide Coated on
Solid Phase (μ titer plates)

+

Sample (1:200)

+

Goat anti-Hu HRPO

Read OD 490 nm

Figure 3: Positive Controls for PEIAs

HIV-1 Group M Control

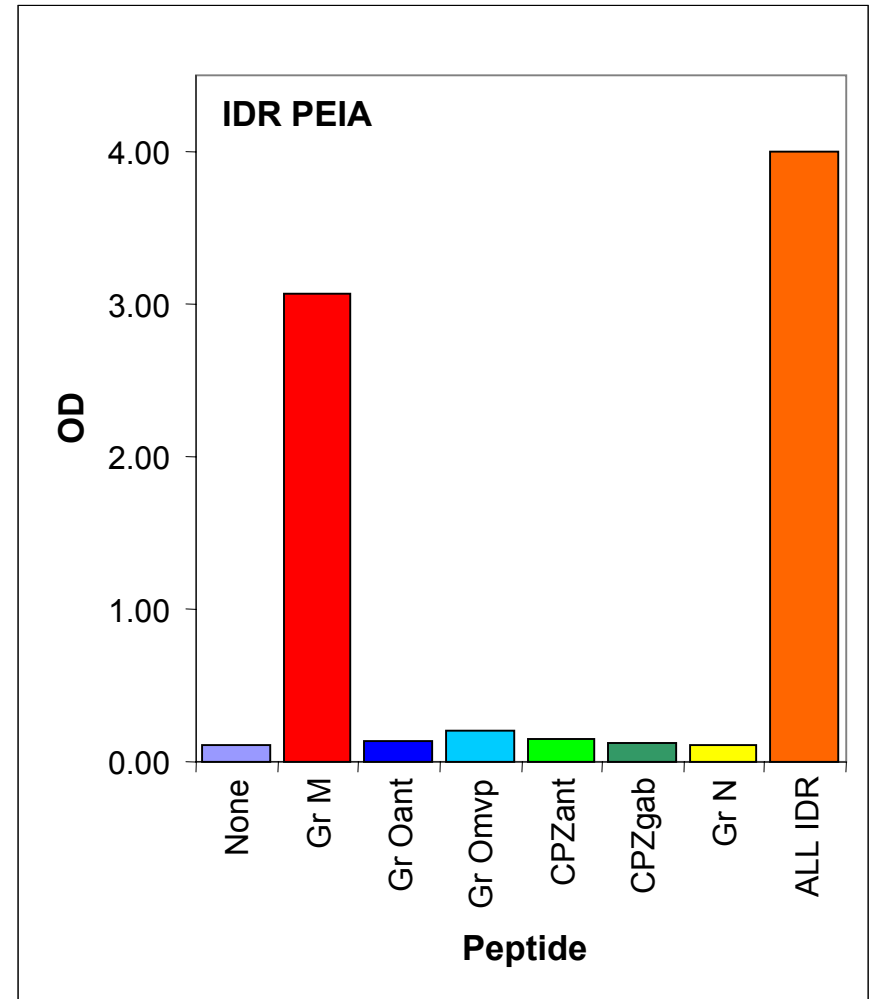
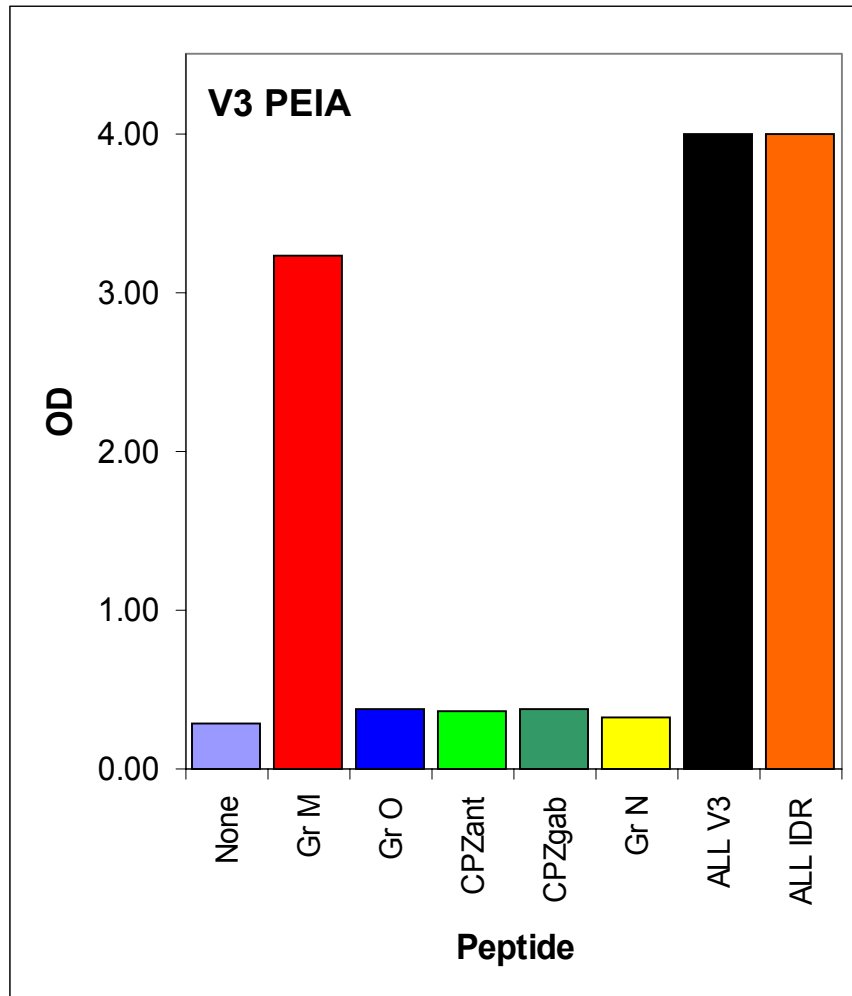


Figure 3: Positive Controls for PEIAs

HIV-1 Group O Control

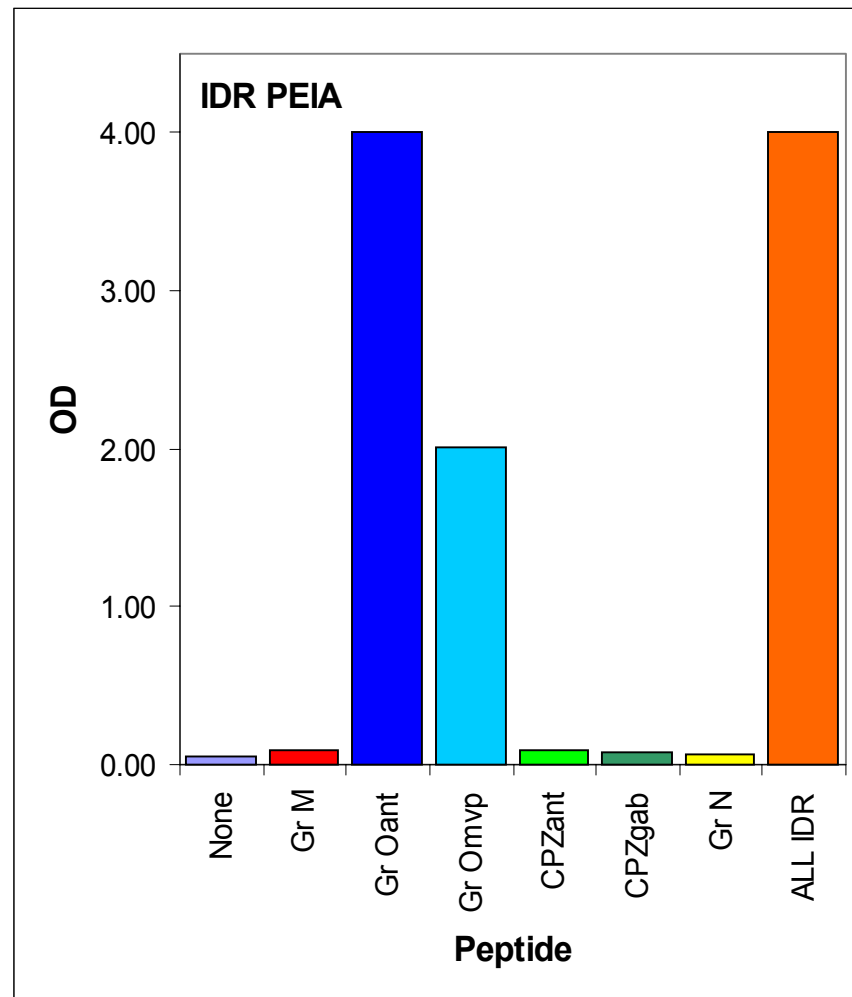
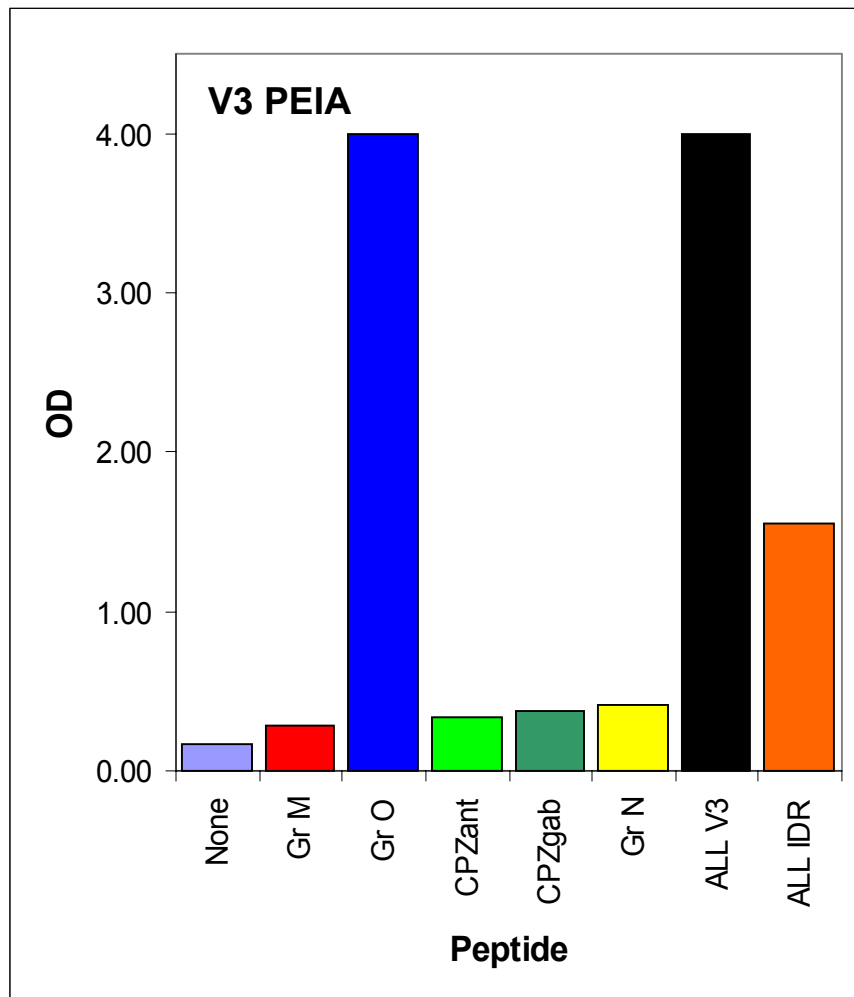


Figure 4: PEIA Results for the HIV-1 Group M/O Dual Infections

- 98CM441 is reactive to both HIV-1 Group M and O in both the V3 and IDR PEIAs.

Specimen 98CM441

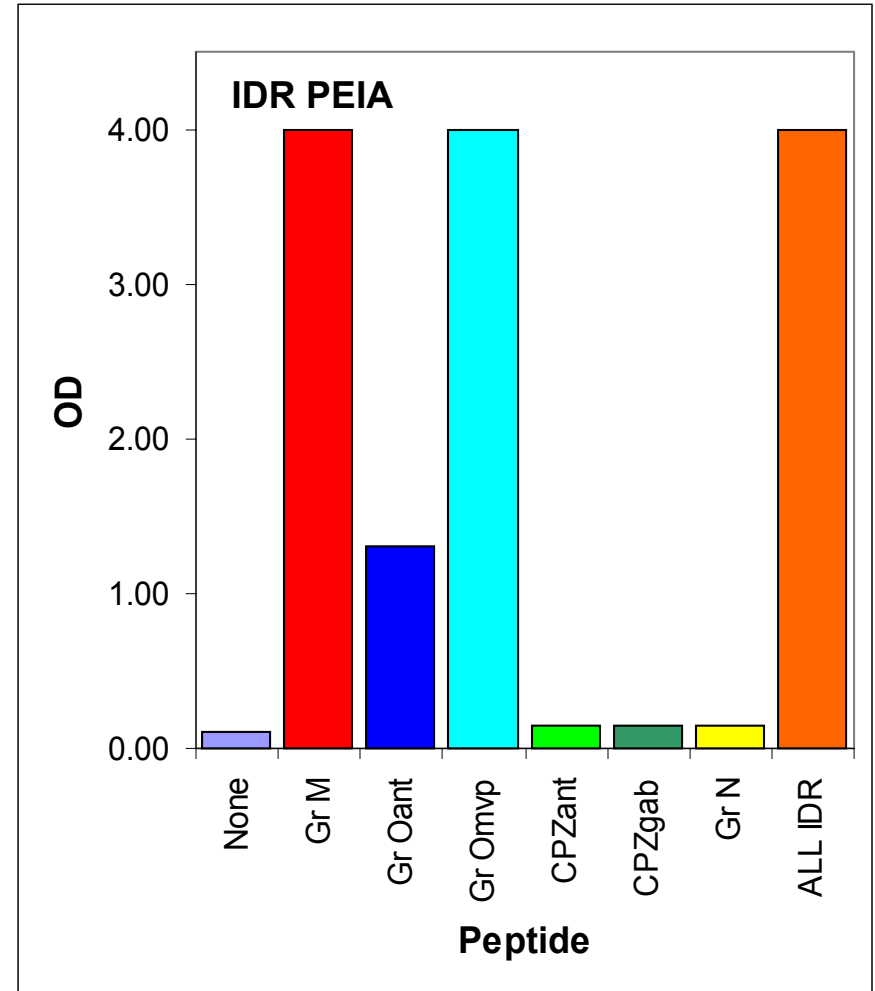
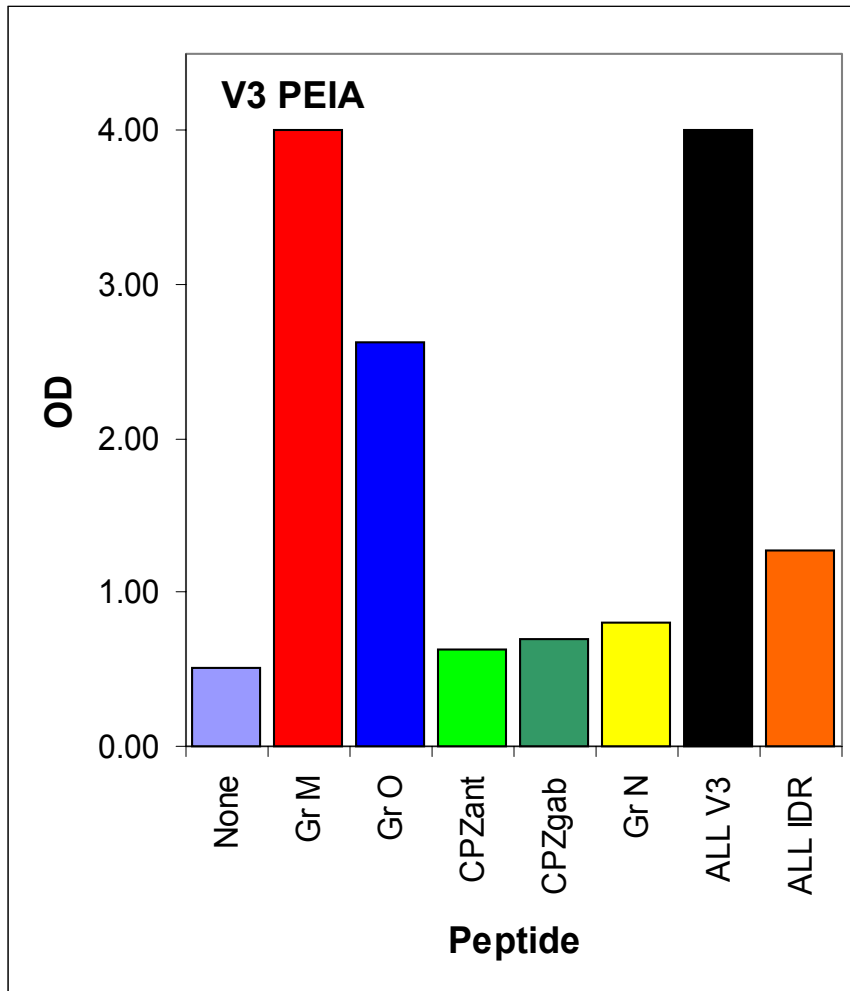


Figure 4: PEIA Results for the HIV-1 Group M/O Dual Infections

- ST-99-0789 is reactive to Group O in the V3 PEIA and Group M in the IDR PEIA.

Specimen ST-99-0789

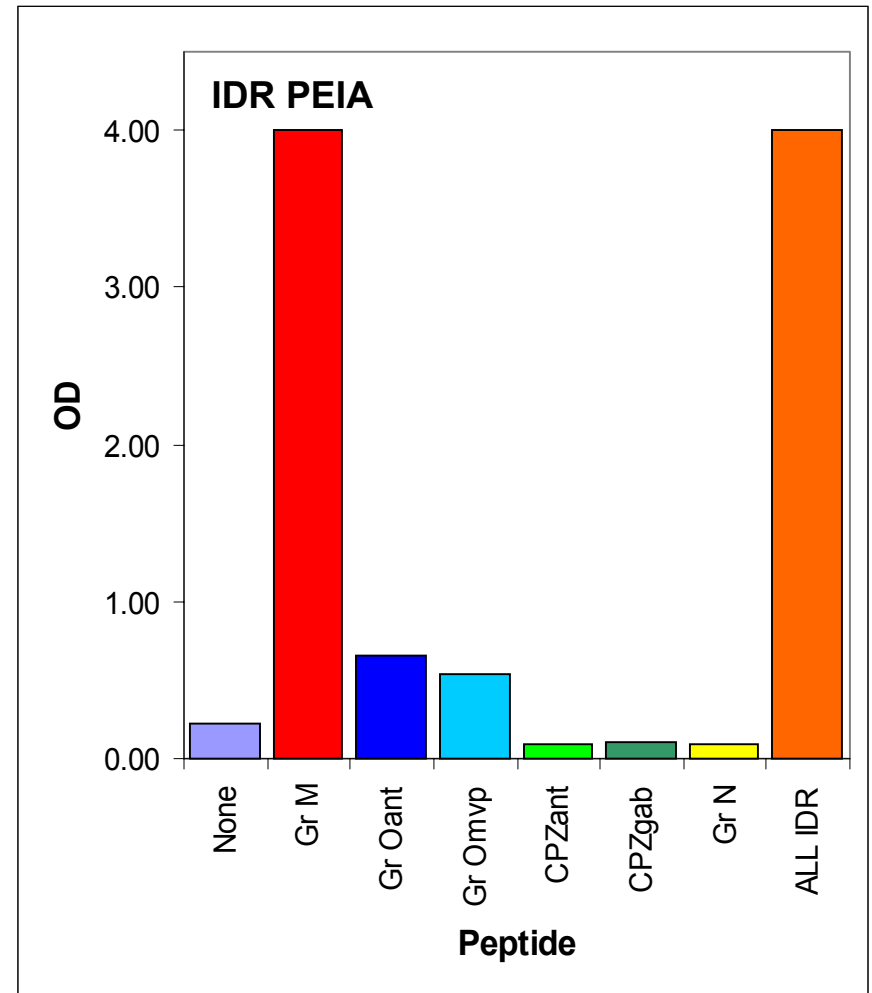
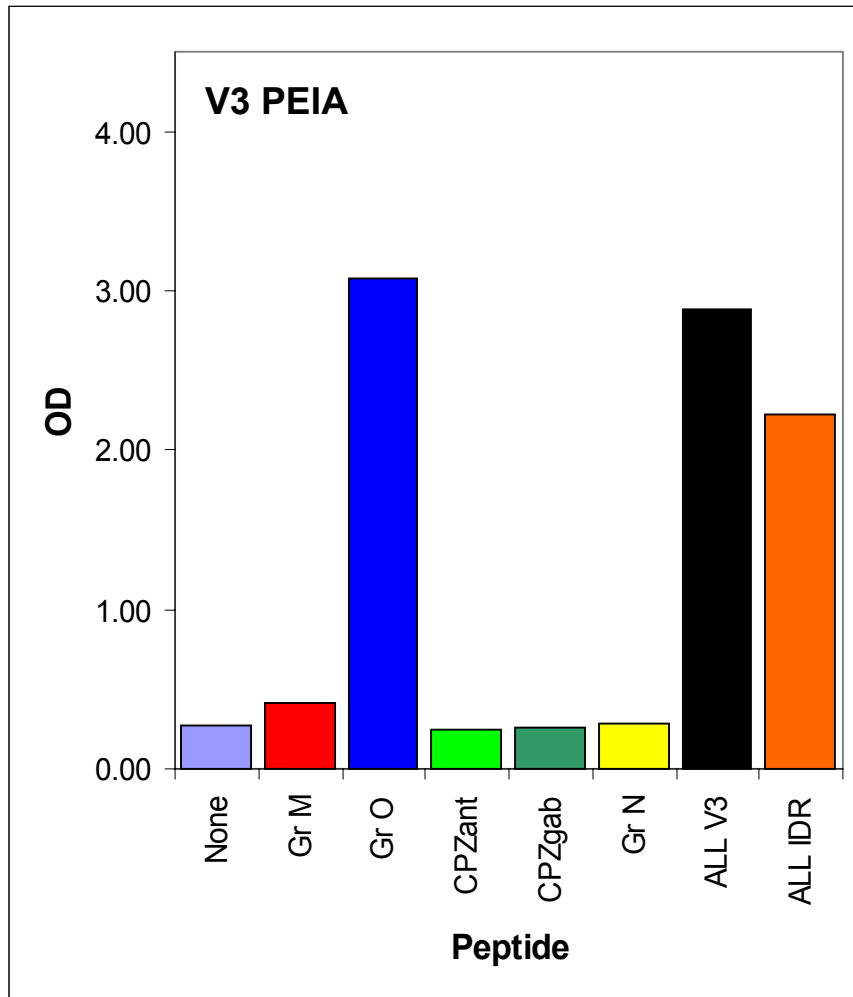


Figure 4: PEIA Results for the HIV-1 Group M/O Dual Infections

- DSC-99-1320 is reactive to Group M in the V3 PEIA and reactive to both Group M and O in the IDR PEIA.

Specimen DSC-99-1320

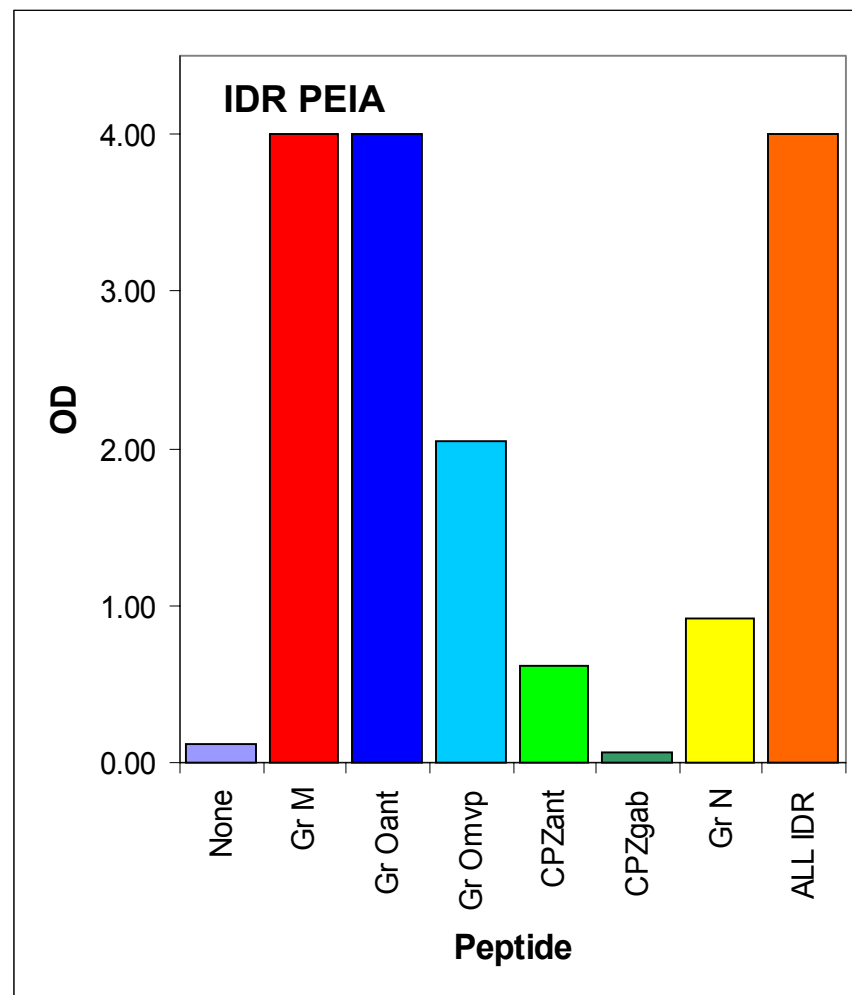
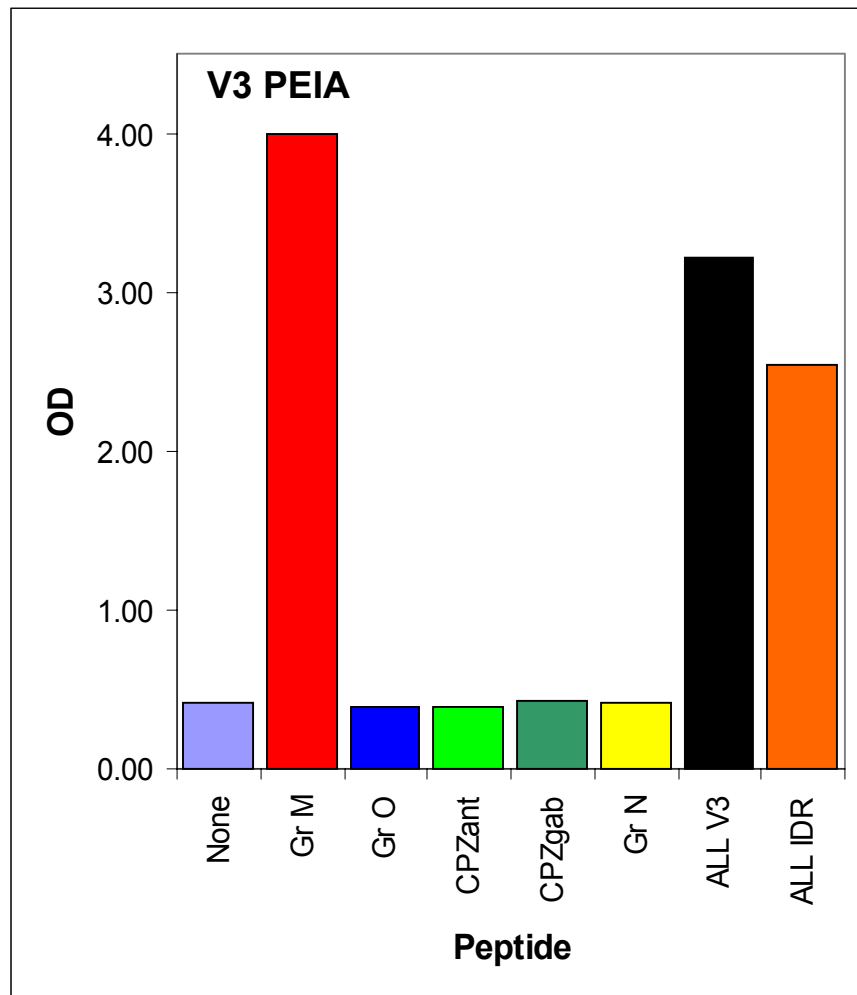


Figure 5: Schematic of HIV-1 Group O Genome Regions Amplified

Genome regions were amplified from plasma by nested RT-PCR using Group O specific primers. For most of the HIV-1 group O isolates, approximately half of the genome (~4000 nts) was sequenced. For 2 of the Group M/O dual infections, 98CM441 and ST-99-0789, only sub-regions of *env* (V3 and IDR) were amplified.

Group O Genome Regions Amplified by PCR

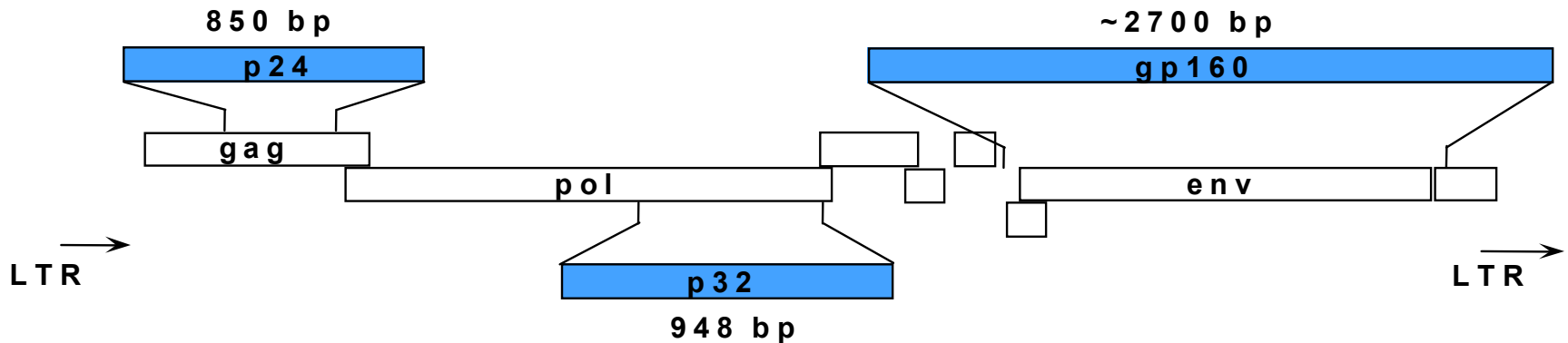


Figure 6: Phylogenetic Trees for HIV-1 Group O Isolates

Sequences were aligned, gap-stripped, and analyzed using the Phylip programs Dnadist, Neighbor, Drawtree, Seqboot, and Consense. Position of outgroup branch (Group M, HXB2) is indicated (■). Reference strains ANT70, MVP5180, and VAU are shown. Bootstrap values (%) are shown for selected branches. Phylogenetic clusters are indicated by Roman numerals with U denoting unclassified.

- The Group O isolates collected in the northwest region of Cameroon are genetically diverse.
- For each Group O isolate, phylogenetic cluster is congruent across the 3 gene segments, *gag*, *pol*, and *env*.

SUMMARY: GROUP O ISOLATES

SPECIMEN ID	Phylogenetic Cluster		
	gag p24	pol p32	env gp160
PHC-00-234	la	lu	lu
SH-99-2859	la	la	la
ST-99-0789	lb	lb	lb*
ST-99-1214	lb	lb	lb
98CM17115	ll	ll	ll
AT-99-0022	lu	lu	lu
AT-99-0138	lu	lu	lu
PHC-00-029A	lu	lu	lu
DSC-99-1320	IV	IV	IV
PHC-00-094A	U	U	U
SH-00-0006	U	U	U
SH-00-0578A	U	U	U
98CM441	V	V	V*
SJG-00-125	negative	negative	negative
* Only env V3 and IDR sub-regions were amplified and sequenced			
Isolates in RED are from M/O dual infections			

Figure 7: **Schematic of HIV-1 Group M Genome Regions Amplified**

Genome regions were amplified from plasma by nested RT-PCR using Group M specific primers.

Group M Genome Regions Amplified by PCR

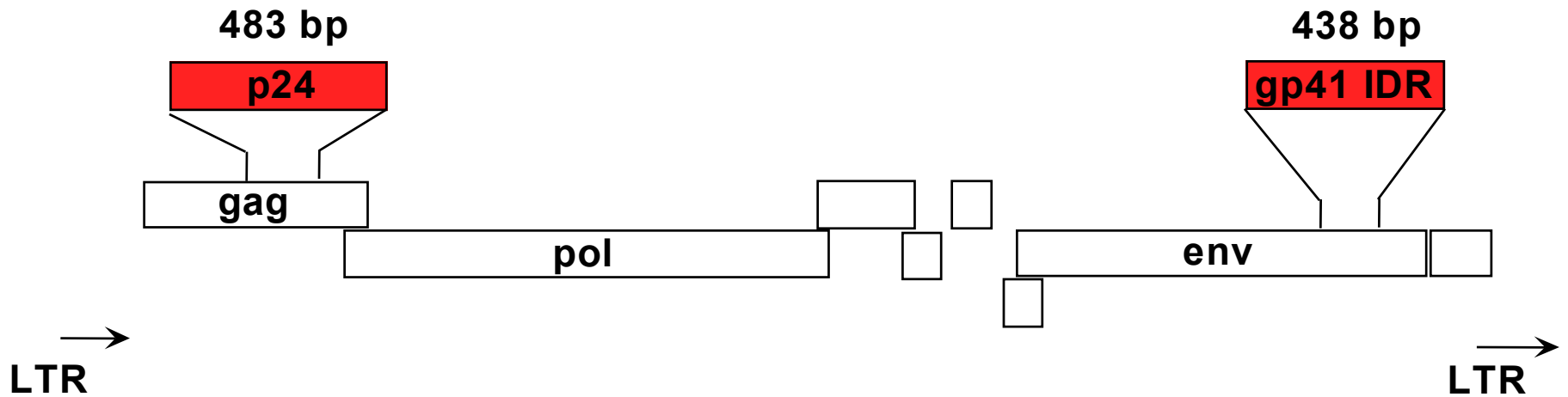


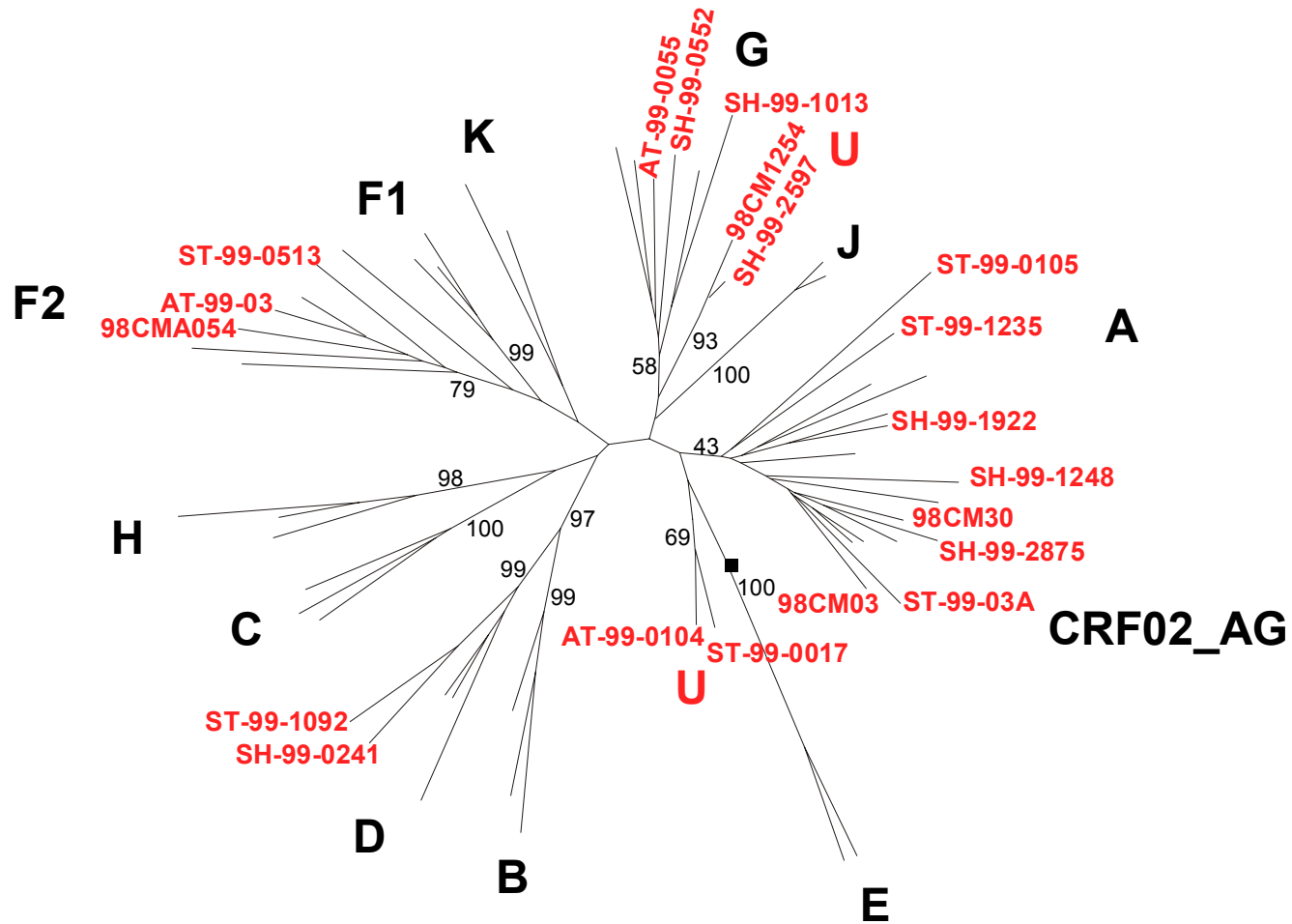
Figure 8: Phylogenetic Trees for HIV-1 Group M Isolates

Analysis is shown for a subset of the sequences obtained from group M isolates. Sequences were aligned, gap-stripped, and analyzed using the Phylip programs Dnadist, Neighbor, Drawtree, Seqboot, and Consense. Position of outgroup branch (Group O, ANT70) is indicated (■). Bootstrap values (%) are shown for selected branches. The subtype branches are labeled.

- The Group M isolates collected in the northwest region of Cameroon are genetically diverse.
- The majority of the isolates fall in the diverse subtype A branch with many clustering with the CRF02_AG reference strains.
- Several isolates are unclassified (U).

Figure 8

HIV-1 Group M Isolates: Phylogenetic Tree *env* gp41 IDR (368 nts)



SUMMARY: GROUP M ISOLATES

SUBTYPE (gag/env IDR)	NUMBER	PERCENTAGE
A	139	83.0%
CRF02_AG [^]	83	60.0%
D	4	2.4%
F2	4	2.4%
G	6	3.6%
MOSAIC*	14	8.4%
TOTAL	167	

[^] Subtype A isolates that clustered with CRF02_AG reference strains in both gag and IDR.

* Mosaics consisted of gag/IDR subtypes:

A/D, A/E, A/F2, A/G, A/U, G/A, G/U

Figure 9: **Schematic of HIV-1 Group M and O Genome Regions Amplified from Dual Infections**

Genome regions were amplified from plasma by nested RT-PCR using Group M specific primers (red) and Group O specific primers (blue).

Group M and Group O Genome Regions Amplified by PCR

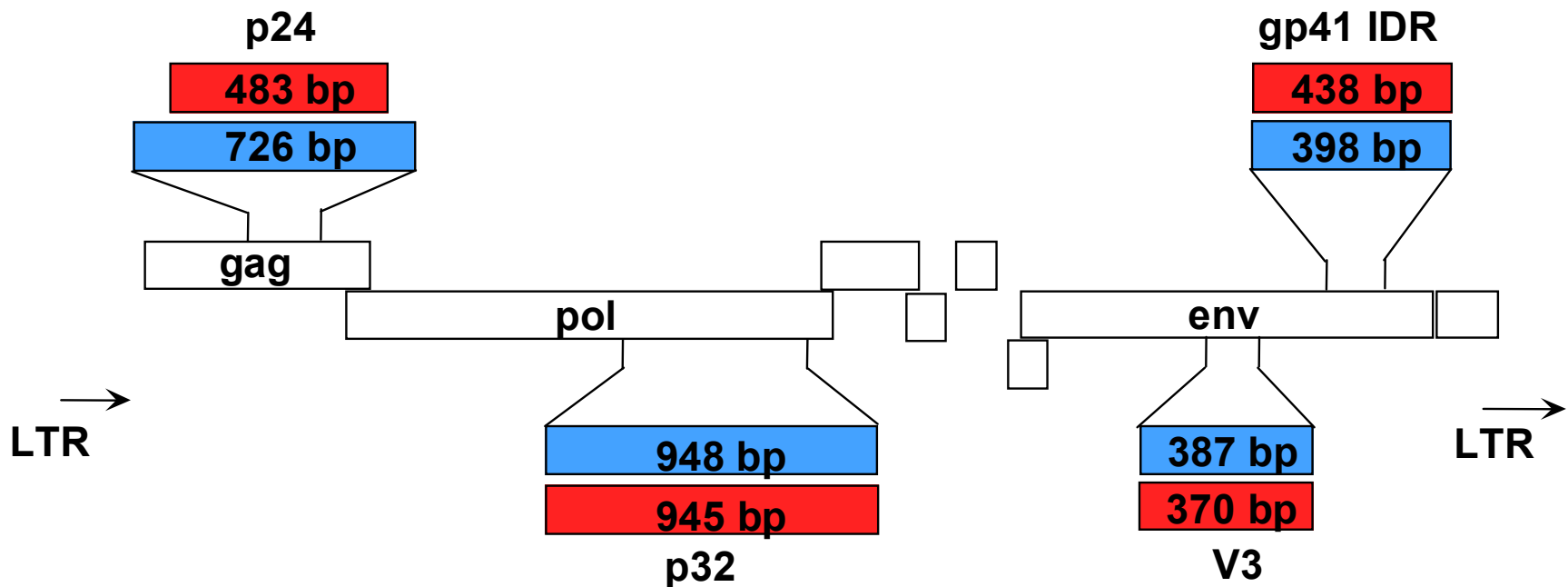


Figure 10: Phylogenetic Trees for HIV-1 Group M/O Dual Infections

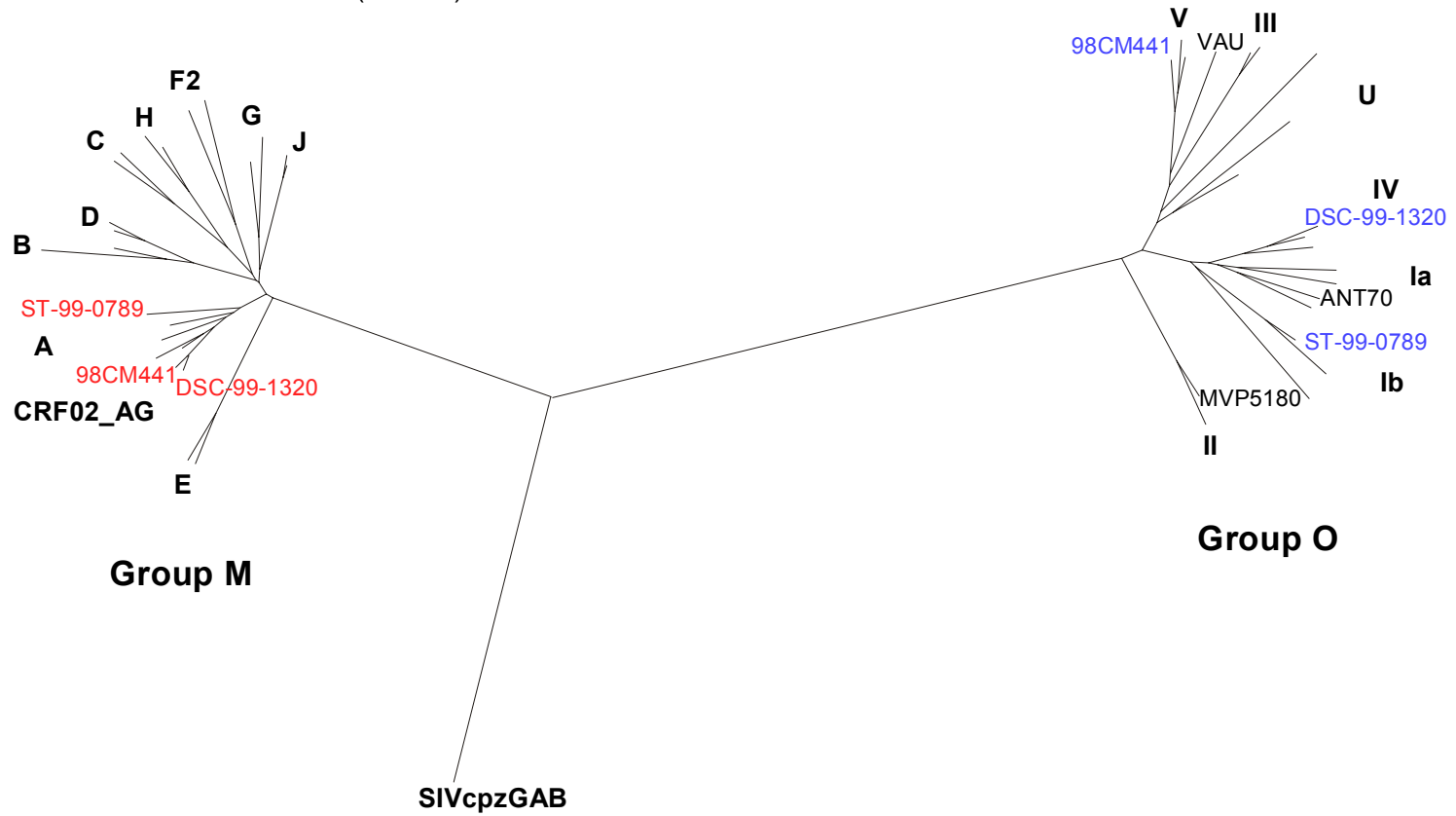
Sequences were aligned, gap-stripped, and analyzed using the Phylip programs Dnadist, Neighbor, Drawtree, Seqboot, and Consense.

- Group M specific primers amplified Group M sequences from each specimen.
- Group O specific primers amplified Group O sequences from each specimen.
- Both Group M and O sequences were amplified from each specimen indicating the presence of at least two viruses.
- Both Group M and O sequences were amplified from the *env* region of DSC-99-1320 but only Group O sequences were amplified from *gag* and *pol* indicating that this specimen may contain a M/O recombinant virus. Longer PCR fragments are needed to determine if any Group M/O recombination has occurred.

Figure 10

HIV-1 Group M/O Dual Infections

env IDR (337 nts)



SUMMARY: GROUP M/O DUAL INFECTIONS

Serology

Specimen ID	MO2N	3A77(S/CO)	PEIA	
			V3	IDR
98CM441	M	8.96	M, O	M, O
ST-99-0789	M	17.39	O	M
DSC-99-132	O	17.70	M	M, O

PCR Amplification

Specimen ID	gag	pol	V3	IDR	
98CM441	M, O	M, O	M, O	M, O	M Subtype CRF02_AG, O Cluster V
ST-99-0789	M, O	M, O	M, O	M, O	M Subtype Unclassified, O Cluster Ib
DSC-99-1320	O	O	M, O	M, O	M Subtype CRF02_AG, O Cluster IV

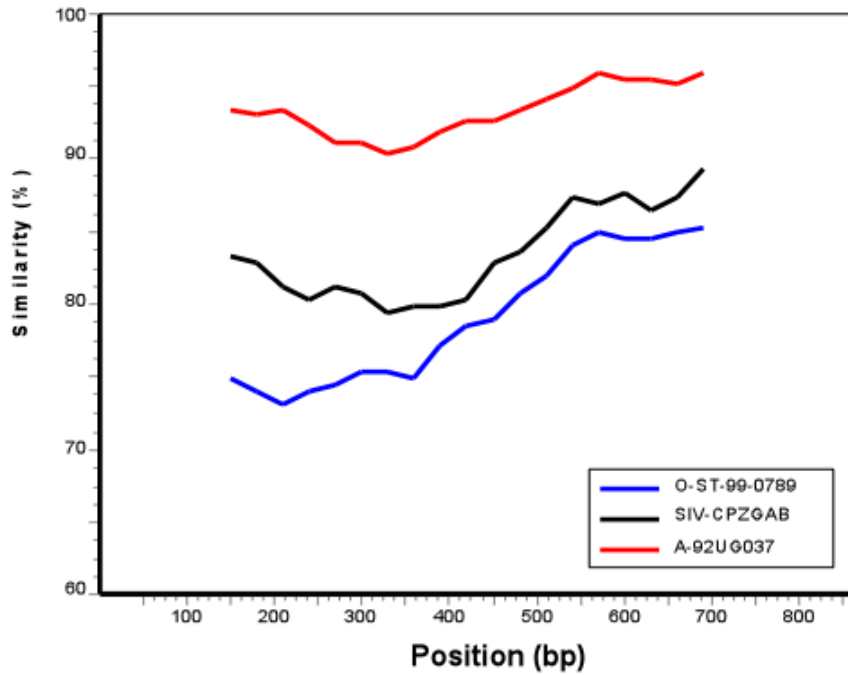
Figure 11: HIV-1 Group M/O Dual Infection – Simplot Analysis *pol* p32

Simplot analysis is shown for the *pol* p32 sequences amplified from specimen ST-99-0789.

- The Group M sequence does not contain segments of Group O.
- The Group O sequence does not contain segments of Group M.
- Simplot analysis of all sequences obtained from the Group M/O dual infections also show a lack of M/O recombination.

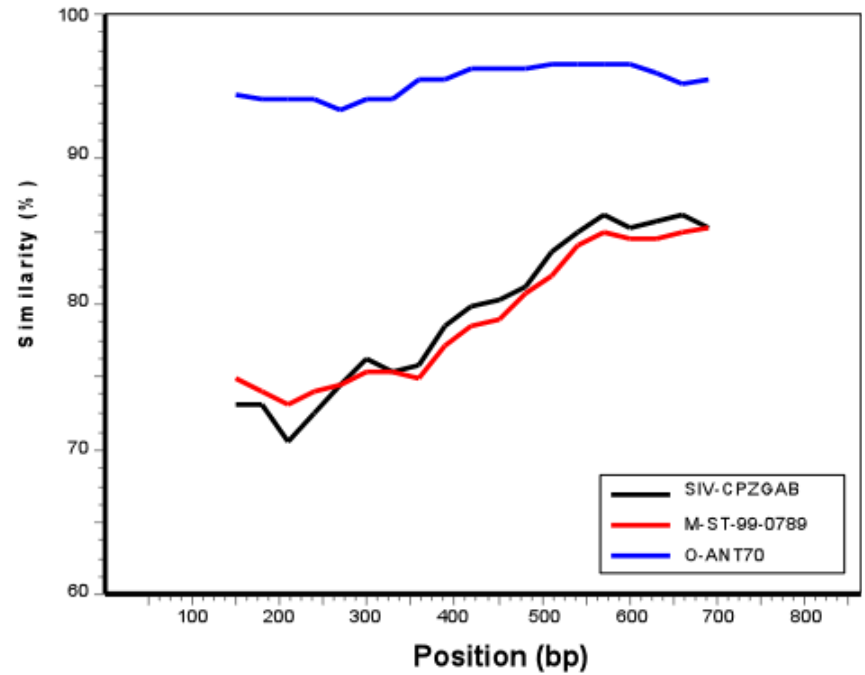
Figure 11

ST-99-0789: Group M



Window: 300 bp, Step: 30 bp, Gap Strip: On, J-C Correction: On

ST-99-0789: Group O



Window: 300 bp, Step: 30 bp, Gap Strip: On, J-C Correction: On

SUMMARY:

- Majority of HIV infections in northwest Cameroon are due to HIV-1 Group M subtype A (by *gag* and *env*).
- Most subtype A isolates cluster with CRF02_AG reference strains.
- Group M inter-subtype mosaics are common; based on *gag* and *env* gene fragments, mosaics represent at least 8% of Group M infections (excluding possible CRF02_AG recombinants).
- The prevalence of Group O infections is low - only 0.45% of total HIV infections.
- Genetic diversity among the Group O isolates is high.
- 3 specimens were identified that are dually infected with HIV-1 Group M and O.