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Diversity within the Immunodominant Epitopes of Envelope gp41 from HIV-1 in Kenya and Its Effects on Performance of the HIV-1 Antibody-Based Detection Kits

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Abstract: *Background*: Human Immunodeficiency Virus (HIV) is characterized by high rates of genetic variability *in vivo* that could affect the performance of the HIV-1 Antibody-Based Detection Kits in use in Kenya. *Objective:* This study aimed at establishing the diversity of Envelope gp41 Epitopes and its effects on performance of the currently used HIV diagnostic kits in Kenya. *Methods:* Two hundred (200) HIV positives and 200 HIV negatives samples were collected from the Regional Blood Transfusion Centers (RBTCs) in Kenya. Viral RNA was extracted from 96 HIV Positive samples and sequenced on gp41-immunodominant region (IDR).The sequences obtained were analyzed using various applications in the Los Alamos HIV Database. The HIV gp41-IDR consensus sequence generated was used to synthesize gp41 IDR peptide. The Global HIV gp41-IDR Consensus nucleotide sequence was obtained from literature and used also to synthesis corresponding gp41 IDR peptide. The two peptides were used to prepare HIV Testing ELISA kits. The 400 plasma samples that had been collected from this study from RBTCs were tested using five HIV testing kits approved for use in Kenya. The same samples were tested using the two ELISA system developed. *Results*: The HIV Consensus gp41-IDR peptide from Kenya displays a similarity of 93.0% against HXB2 sequence and 95.3% against Global HIV Consensus gp41-IDR amino acid sequence. There were 331 (7.8%) substitutions in the Consensus gp41-IDR peptides (Kenya) out of which 151substitutions were due to the substitution in positions $A^{96}\rightarrow N$ (n = 79) and $A^{101}\rightarrow S$ (n = 75). Both Consensus gp41-IDR peptides (Kenya) and Consensus gp41-IDR peptides (Global) showed common substitutions rate of 4.2% (n = 151). All the kits that were used showed 100% agreement in results. *Conclusion*: Although the HIV Consensus gp41-IDR peptides (Kenya) showed marked substitutions in respect to Consensus gp41-IDR peptide (Global) there was no difference in effects on the performance of the HIV-1 Antibody-Based Detection Kits in use in Kenya.

Keywords: Diversity, HIV, Substitution, Consensus, Immunodominant Region, gp41

1. Introduction

Globally, more than 35 million people are living with HIV/AIDS with the global incidence being estimated at 3 million (1). According to the Kenya AIDS Indicator Survey (2012), the prevalence of HIV in Kenya among adults 15-64 years old was then estimated to be 5.6% (2).The Human Immunodeficiency Virus (HIV) is characterized by high rates of genetic variability *in vivo* due to rapid viral turnover in a patient with an active disease (about 10^{10} viral particles per day) and lack of proof-reading mechanisms during reverse transcription that produces high mutation rates of about 10^{-4} mutations per nucleotide (3). Mutation rates and selection rates vary for different components of the HIV genome with *gag* and *pol* genes being less variable than the *env* gene, which has been estimated to be 1% per year. This mutation rate allows the virus to evade host immune responses. Studies have shown a changing trend of HIV 1 subtypes in Nairobi, Kenya, where subtype A1is the most predominant subtype at 65% (3). Different HIV strains are known to recombine to produce large genetic alterations that could, at times, produce

genetic shifts (4,5, 6).

The *env* gene encode for gp160 that is enzymatically cleaved into two peptides gp120 and gp41. The gp41 contains the Immunodominant region (IDR) in the amino-terminal portion and more than 99% of HIV-1infected individuals produce antibodies directed to this region. The IDR of gp41 contains the following sub-regions the CTL epitope(aa 71 to 82;AVERYLKDQQLL) and the Cysteine Loop (aa 87 to 93; CSGKLIC)and ectodomain region (aa 151 to 156; ELDKWA) (13, 14). However, gp41 peptide fragment without the ectodomain region has been found to still detect more than 99% of HIV infected cases (9).

It has been shown that some HIV Antibody-Based diagnostic kits that have been developed using multisubtype gp41 recombinant antigen (rIDR-M) were highly reactive with HIV antibodies in sera from different subtypes and equivalently detected antibodies to divergent subtypes B and AE from Thailand, in contrast to individual gp41 peptides derived from respective subtypes (7). In several assays designed to test for HIV Incidence the accuracy of the assays was found to be affected by the viral subtypes(15, 16).

In a study carried out by Thorstensson *et al* (1998) the performance of 14 commercially available HIV-1/2 antibody assays were compared with: HIV-negative blood donor sera from Sweden, unselected blood donor and patient sera from Tanzania; unselected sera from outpatient clinics in Guinea-Bissau; HIV-1 antibody positive sera from Sweden and Tanzania and HIV-2 antibody positive sera from Guinea-Bissau. The results of this study showed that 12 (85.7%) of the 14 assays identified correctly all HIV-1 and HIV-2 antibody positive sera. One Tanzanian HIV-1 antibody positive sample with complete banding pattern on Western blot was not detected by two of the ELISAs employing synthetic peptides. The assays showed a high specificity ranging from 99.2 to 100% when used for analysis of Swedish blood donor sera, while most of the assays showed a significantly lower specificity, 91.9-99.6%, when used for testing African specimens (8).

However some studies have established that consensus gp41-IDR from group M peptides (WGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTA VPWNASW) was able to detect all 130 group M sera (10 subtype A, 21 subtype B, 13 subtype B9, 20 subtype C, 21 subtype D, 14 subtype E, 25 subtype F, and 6 subtype G) resulting in a test sensitivity of 100% but a showed less cross-reactivity with group O peptides of 84% (9). Despite the high genetic divergence between HIV-1 groups M and N, all group N infections have been detected using five commercial HIV immunoassays (10).

With such divergent results of various studies on the effect of diversity HIV on performance of the HIV-1 Antibody-Based Detection Kits in other parts of the world it was necessary to establish the current situation in Kenya.

2. Materials and Methods

Sampling: Four hundred blood samples, 200 HIV positives and 200 HIV negatives, were collected from the Regional Blood Transfusion Centers (RBTCs) in Kenya (Nairobi, Nakuru, Kisumu, Embu and Mombasa) over a period of three months (May to July 2011) after having removed donors' identifiers.

PCR amplification, sequencing, sequence- analysis and peptide synthesis: HIV viral RNA was extracted from 96 HIV Positive samples using the QIAamp viral RNA kit (Qiagen, Valencia, Calif.) according to the manufacturer's protocol. For reverse transcription (RT) and primary PCR, the primers used were GP40F1 (forward; 5′TCTTAGGAGCAGCAGGAAGCACTATGGG) and GP41R1 (reverse;5′AACGACAAAGGTGAGTATCCCTGCCTAA). For the nested PCR, the primers used were GP46F2 (forward; 5′ACAATTATTGTCTGGTATAGTGCAACAGCA) and GP47R2 (reverse; 5′TTAAACCTATCAAGCCTCCTACTATCATTA). Three to 10 µl of the RNA extract were used to synthesize cDNA with primer GP41R1 (20 µM) and the GeneAmp RNA PCR kit(Perkin-Elmer Cetus, Norwalk, Conn.) following the manufacturer's protocol. The 20-µl cDNA reaction mixture was then added to a PCR mixture containing 50 µM GP40F1 and 30 µM GP41R1, 1× GeneAmp PCR buffer II, 1.25 mM MgCl2, and 2.5U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Foster City, Calif.) and brought to a final volume of 100 µl with sterile distilled water. After initial denaturation at 94°C for 2 min, 35 cycles of PCR were performed in the GeneAmp 9600 thermo cycler (Perkin-Elmer Cetus, Norwalk, Conn.). Each cycle consisted of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 5 min. For nested PCR, 5 µl of the primary PCR product were added to a 100-µl PCR mixture containing reagents similar to those in the primary PCR, except that the primers were replaced by 25 µM each GP46F2 and GP47R2. The PCR mixture was subjected to 35 cycles under the same conditions as the primary PCR. After PCR, the nested PCR products were electrophoresed in 1.5% agarose gels along with a 100-bp ladder (Gibco, Grand Island, N.Y.) and visualized under UV light by ethidium bromide staining. The Complementary DNA was sent to Macrogen Europe (Amsterdam, Netherlands) for sequencing using use of a big dye terminator v. 3.1 (PE) and the same primers that were used for initial nested PCR (GP46F2 and GP47R2). The resultant sequences were aligned using CLUSTALW program within MEGA 4 Data Program (Tamura *et al.,* 2007). The prevailing HIV consensus sequence was generated by use of the software "Advanced Consensus Maker" in the Los Alamos Database (Los Alamos Database, 2009) using aligned sequences. The Global Consensus was obtained from literature (10). The Consensus sequence of gp41 IDR established in this study (Kenya) and the Global Consensus gp41 IDR were then sent to LifeTein LLC (Hillsborough, NJ) for synthesis of gp41 IDR peptides.

Development of ELISA Test Kit for using synthetic peptide derived from the gp41 region representing the

consensus sequence for HIV in Kenya. The two synthetic gp41IDR peptides (Kenya: WGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTN VPWNSSW and Global: WGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTA VPWNASW) were used to develop an Enzyme-Linked Immunosorbent Assays (ELISA) as described previously. Briefly, polyvinylplates (ImmulonII; Dynatech Laboratories, Inc., Alexandria, VA.) were coated with 5ug of synthetic peptide per ml (100 ml/well) in 0.01 M carbonate buffer(pH9.6)andincubatedovernightat4°C.Theplateswerewashedsixtimes with phosphate-buffered saline containing 0.05% Tween 20 (PBST); excess reactive sites wereblockedbytheadditionof5%bovineserumalbumininphosphate-buffered saline - 0.05% Tween 20. This step was followed by the addition of a 1:100 dilution of each test serum. The plates were incubated overnight at 4°C. After six more washes, Fc-specific, alkaline phosphatase-conjugated goat antibody to human immunoglobulin G (Sigma, St. Louis, Mo.) was added, and the plates were left at room temperature for 1hr. This step was followed by washing six times with PBST. This step was then followed by addition of 100µl of 3,3',5,5'-Tetramethylbenzidine / hydrogen peroxide substrate(*MP* Biomedical, Santa Ana, Calif.)and 30 minutes incubation in the dark; stopping the reaction by adding 100µl of 1M sulfuric acid(Sigma, St. Louis, Mo.); and measuring the O.D at 450nm with Multiscan ELISA Reader (Thermo Fisher Scientific, Waltham, MA). The cutoff values were calculated by adding 0.1 to the mean optical densities plus 3 standard deviations of normal control sera in the assay. The ELISA was optimized through a series of experiments.

Testing of the HIV positive and negative samples: The400 plasma samples that had been collected in this study from RBTCs were tested using the following HIV testing kits approved for use in Kenya after prequalification by World Health Organization (WHO):Samples were tested using the following antibody-based HIV diagnostic kits: (HIV (1 + 2) Antibody (colloidal gold) (KHB Shanghai Kehua Bio-engineering Co, Shanghai, China), First Response™ 1-2.0 (PMC Medical Pty. Ltd, Daman, India), Uni-Gold™ HIV test (Trinity Biotech, USA); Determine™ HIV-1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands), Alere Determine™ HIV-1/2 Ag/Ab Combo (Orgenics Ltd, Yavne, Israel), Aware™ HIV-1/2 BSP (Calypte Biomedical Corporation, Oregon, USA) and Vironostika™ Uni-Form II Ag/Ab ELISA (bioMérieux, Marcy-l'Etoile, France). The same samples were tested using the two ELISA system developed in this study.

3. Results

Relative to HXB2 Los Alamos prototype sequence the HIV Consensus gp41-IDR peptides from Kenya displays three amino acid substitutions where I^{70} →V, A^{96}→N and A^{101}→S (93.0% similarity) and only two amino acid substitutions relative to HIV Consensus gp41-IDR peptide (Global) where A^{96}→N and A^{101}→S (95.3% similarity) *(Fig 1)*

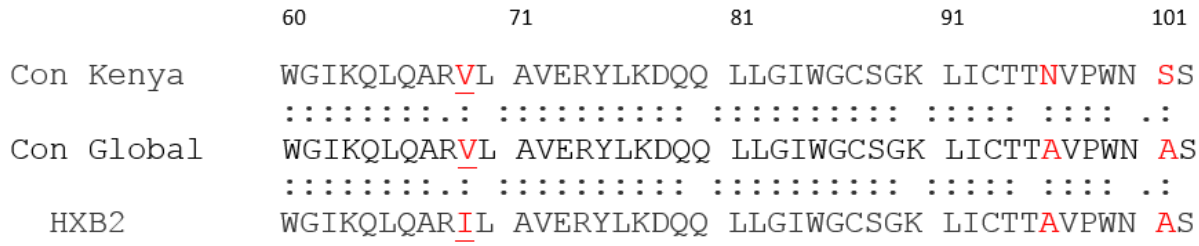

Figure 1. *Alignment of Consensus gp41-IDR peptides (Kenya, global and HXB2).*

There was 100% sequence similarity in the key sub-regions of IDR of gp41: the CTL epitope (aa 71 to 82;AVERYLKDQQLL) and the Cysteine Loop (aa 87 to 93; CSGKLIC). This implies that the previously named substitutions within IDR of gp41 could generally have minimal effects on immunogenicity of the peptides.

Distribution of subtypes within the samples collected A (87%), C (5.4%), D(5.4%) and others (2.2%)

Diversity of HIV gp41-IDR of individual samples

When the individual sequences in the study were analyzed for the substitutions in relation to the HIV Consensus gp41-IDR peptide (Global) the following substitutions were obtained (*Table 1*):

Table 1. *Diversity of HIV gp41-IDR of individual samples.*

HIV Sequence Identity	CTL Epitope	Cysteine Loop	Other regions	HIV testing Identity and results ("+" for positive results and "-" for negative results							
				1	2	3	4	5	6	7	8
KPDR_004(A1)	K^{77}→R	S^{88}→A; K^{90}→V; L^{91}→I	60-101(del 96; Ins(93a = P) A^{101}→S	+	+	+	+	+	+	+	+
KPDR_010(A1)	K^{77}→E; D^{78}→H	0	A^{96}→N; v^{69}→L A^{101}→S	+	+	+	+	+	+	+	+
KPDR_011(A1)	R^{68}→S; K^{77}→R	L^{91}→H	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+

HIV Sequence Identity	CTL Epitope	Cysteine Loop	Other regions	HIV testing Identity and results ("+" for positive results and "-" for negative results							
				1	2	3	4	5	6	7	8
KPDR_014 (A1)	0	L^{91}→H	A^{96}→T	+	+	+	+	+	+	+	+
KPDR_017 (A1)	V^{72}→L	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_019 (A1)	R^{68}→S; K^{77}→R	L^{91}→H	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_047 (A1)	V^{72}→L	0	A^{96}→T; A^{101}→Y	+	+	+	+	+	+	+	+
KPDR_049 (A1)	K^{77}→Q	0	A^{96}→T A^{101}→S	+	+	+	+	+	+	+	+
KPDR_057 (D)	R^{68}→S; K^{77}→R	0	G^{83}→A; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_063 (A1)	Q^{79}→R	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_069 (A1)	V^{72}→L	K^{90}→R L^{91}→I	A^{96}→N; T^{94}→P A^{101}→S	+	+	+	+	+	+	+	+
KPDR_078(A2.CY.94CY017_41	K^{77}→Q	0	A^{96}→F; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_088 (A1)	K^{77}→R	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_095(A1)	0	0	A^{96}→T; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_096 (A1)	K^{77}→V	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_099_15_2_GP46F.ab1(A1)	A^{71}→G V^{72}→L	L^{91}→I; I^{92}→L T^{94}→P	I^{84}→F; A^{96}→F R^{99}→W; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_101A1)	K^{77}→R	L^{91}→I; T^{94}→P	A^{96}→N; A^{101}→S A^{101}→S	+	+	+	+	+	+	+	+
KPDR_110 (A1)	0	0	I^{84}→L; A^{96}→N	+	+	+	+	+	+	+	+
KPDR_125 (A1)	V^{72}→L; K^{77}→V	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_130 (A1)	0	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_133(A2.CY.94CY017_41)	K^{77}→Q	0	A^{96}→F; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_140 (A1)	K^{77}→R	K^{90}→R	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_141 (A1)	A^{71}→G; R^{68}→S K^{77}→R	L^{91}→H	V^{77}→I; I^{84}→V A^{96}→T; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_0146 (A1)	K^{77}→R	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_151 (A1)	0	0	I^{84}→L; A^{96}→T T^{94}→S; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_152 (A1)	0	0	T^{94}→P; A^{96}→N A^{101}→S	+	+	+	+	+	+	+	+
KPDR_155 (A1)	0	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_1561(A2.CY.94CY017_41)	R^{68}→S	0	C^{87}→S;A^{101}→S	+	+	+	+	+	+	+	+
KPDR_162 (A1)	0	0	A^{96}→N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_164 (A1)	K^{77}→R	0	I^{84}→L; A^{96}→N	+	+	+	+	+	+	+	+
KPDR_176 (A1)	V^{72}→L; K^{77}→R	0	A^{96}→N	+	+	+	+	+	+	+	+
KPDR_177 (C)	V^{72}→I	0	Q^{66}→K; A^{67}→T T^{94}→P	+	+	+	+	+	+	+	+
KPDR_179 (A1)	V^{72}→L; K^{77}→R	L^{91}→I	A^{96}→N; A^{101}→T	+	+	+	+	+	+	+	+
KPDR_181(A1)	K^{77}→R	0	A^{96}→ N	+	+	+	+	+	+	+	+
KPDR_185 (A1)	0	L^{91}→H; K^{90}→R	I^{62}→V; V^{69}→I A^{96}→T; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_188_11_07_GP46F.ab1(A1)	K^{77}→R	0	A^{96}→ N	+	+	+	+	+	+	+	+
KPDR_194 (A1)	0	0	Q^{66}→H; A^{96}→ N A^{101}→S	+	+	+	+	+	+	+	+
KPDR_198 (A1)	0	0	Q^{66}→R; A^{96}→ N A^{101}→S	+	+	+	+	+	+	+	+
KPDR_199(D)	R^{68}→S	0	V^{69}→I; I^{84}→L A^{101}→S	+	+	+	+	+	+	+	+
KPDR_200 (A1)	V^{72}→L; R^{68}→S K^{77}→R	0	A^{96}→ N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_202 (A1)	0	0	A^{96}→T	+	+	+	+	+	+	+	+
KPDR_205 (A1)	0	0	L^{70}→ Q; I^{84}→L A^{96}→T; A^{101}→S	+	+	+	+	+	+	+	+

HIV Sequence Identity	CTL Epitope	Cysteine Loop	Other regions	HIV testing Identity and results ("+" for positive results and "-" for negative results							
				1	2	3	4	5	6	7	8
KPDR_212 (C)	0	0	$A^{67}\rightarrow T$; $T^{94}\rightarrow P$ $A^{101}\rightarrow T$	+	+	+	+	+	+	+	+
KPDR_216 (A1)	0	0	$A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_223 (A1)	0	$K^{90}\rightarrow R$; $L^{91}\rightarrow H$	$I^{62}\rightarrow V$; $V^{69}\rightarrow I$ $A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_224 (A1)	$K^{77}\rightarrow Q$	0	$V^{69}\rightarrow M$; $A^{96}\rightarrow T$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_230 (A1)	0	0	$A^{96}\rightarrow N$; $I^{84}\rightarrow L$	+	+	+	+	+	+	+	+
KPDR_235_26_09_GP46F.ab1	$K^{77}\rightarrow R$		$A^{96}\rightarrow N$; $T^{94}\rightarrow A$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_236 (A1)	$Y^{75}\rightarrow F$	0	$A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_237 (A1)	0	0	$V^{70}\rightarrow L$; $I^{84}\rightarrow L$ $A^{96}\rightarrow T$; $A^{101}\rightarrow T$	+	+	+	+	+	+	+	+
KPDR_88 (A1)	$K^{77}\rightarrow G$	0	$A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_96 (A1)	$V^{72}\rightarrow L$; $K^{77}\rightarrow V$	0	$A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_95 (A1)	0	0	$A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_110 (A1)	$L^{81}\rightarrow I$	0	$I^{84}\rightarrow L$; $A^{96}\rightarrow T$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_146 (A1)	$K^{77}\rightarrow R$	0	$A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_151(A1)	0	0	$I^{84}\rightarrow L$; $T^{94}\rightarrow S$ $A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_177 (C)	$V^{72}\rightarrow I$	0	$A^{67}\rightarrow T$; $T^{94}\rightarrow S$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_199 (D)	$R^{68}\rightarrow S$	0	$V^{69}\rightarrow I$; $I^{84}\rightarrow L$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_202 (A1)	0	0	$A^{96}\rightarrow T$	+	+	+	+	+	+	+	+
KPDR_207 (D)	$A^{71}\rightarrow G$; $Q^{79}\rightarrow H$ $L^{81}\rightarrow P$; $L^{82}\rightarrow P$	$C^{93}\rightarrow S$	$T^{94}\rightarrow P$; $A^{96}\rightarrow N$	+	+	+	+	+	+	+	+
KPDR_216 (C)	$R^{68}\rightarrow K$; $K^{77}\rightarrow R$ $L^{81}\rightarrow I$	0	$G^{83}\rightarrow R$; $A^{96}\rightarrow T$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPDR_237 (A1)	$L^{70}\rightarrow Q$	0	$I^{84}\rightarrow L$; $A^{96}\rightarrow T$ $A^{101}\rightarrow T$	+	+	+	+	+	+	+	+
KPBM_258 (A1)	$K^{77}\rightarrow R$	$K^{90}\rightarrow R$; $L^{91}\rightarrow P$	$T^{94}\rightarrow P$ 60-101(del 96)	+	+	+	+	+	+	+	+
KPBM_262 (A1)	$K^{77}\rightarrow T$; $Q^{80}\rightarrow R$	0	$A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPBM_263 (A1)	$R^{74}\rightarrow G$; $K^{77}\rightarrow R$	0	$A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPBM_264 (A1)	$R^{74}\rightarrow G$; $K^{77}\rightarrow R$	0	$A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPBM_310 (A1)	$A^{71}\rightarrow G$; $K^{77}\rightarrow R$	0	60-101(del 63) $A^{96}\rightarrow N$	+	+	+	+	+	+	+	+
KPBM_314 (A1)	$K^{77}\rightarrow R$	0	$A^{96}\rightarrow T$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPBM_324_GP46F.ab1 (A1)	$V^{72}\rightarrow L$	0	$A^{96}\rightarrow N$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPD_710 (A1)	$K^{77}\rightarrow R$	0	$T^{94}\rightarrow P$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPD_721 (A1)	$V^{72}\rightarrow I$; $R^{74}\rightarrow A$	0	$A^{96}\rightarrow S$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPD_724 (A1)	60-101(del 71)	0	Ins(68a = S, 68b = P) A96→ N; A101→S	+	+	+	+	+	+	+	+
KPD_738 (A1)	0	0	$A^{96}\rightarrow N$	+	+	+	+	+	+	+	+
KPD_764 (A1)	$V^{72}\rightarrow L$; $K^{77}\rightarrow R$		$A^{96}\rightarrow N$; $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPD_830 (A1)	A71→G		$V^{69}\rightarrow I$ $I^{84}\rightarrow L$ $A^{96}\rightarrow N$ $A^{101}\rightarrow S$	+	+	+	+	+	+	+	+
KPD_846_GP46F.ab1(A1)	60-101(del 75)	0	A96→ N; Q66→ P A96→ N; R68→E Ins(66a = G, 68a = S) 60-101(del 70)	+	+	+	+	+	+	+	+

HIV Sequence Identity	CTL Epitope	Cysteine Loop	Other regions	HIV testing Identity and results ("+" for positive results and "-" for negative results							
				1	2	3	4	5	6	7	8
KPD_860 (A1)	K^{77}→R	O	I^{84}→L; A^{96}→ N A^{101}→S	+	+	+	+	+	+	+	+
KPD_982 (A1)	K^{77}→R	0	A^{67}→ T; A^{96}→ N A^{101}→S	+	+	+	+	+	+	+	+
KPD_1023 (D)	K^{77}→R	L^{91}→H	V^{69}→I; I^{84}→L A^{96}→ N; A^{101}→S	+	+	+	+	+	+	+	+
KPD_1034 (A1)	K^{77}→R	0	A^{96}→ N	+	+	+	+	+	+	+	+
KPD_1156 (A1)	0	0	A^{96}→ N; A^{101}→V	+	+	+	+	+	+	+	+
KPD_1168 (C)	V^{72}→L; K^{77}→R	L^{91}→R	T^{94}→P; P^{98}→R A^{101}→S	+	+	+	+	+	+	+	+
KPD_1178 (A1)	R^{74}→K	0	A^{96}→ N; T^{94}→P C^{87}→L; A^{101}→S	+	+	+	+	+	+	+	+
KPD_1216 (A1)	V^{72}→L	R^{74}→K	A^{96}→ N; T^{94}→P C^{87}→L; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_151 (A1)	0	0	I^{84}→L; A^{96}→ T; T^{94}→S; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_194 (A1)	V^{72}→L	0	A^{96}→ N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_199 (D)	R^{74}→S	0	V^{69}→I; I^{84}→L A^{101}→S	+	+	+	+	+	+	+	+
KPDR_202 (A1)	K^{77}→R	0	A^{96}→ T; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_288 (A1)	V^{72}→L	0	A^{96}→ N; A^{101}→S	+	+	+	+	+	+	+	+
KPDR_289 (A1)	0	0	A^{96}→ T	+	+	+	+	+	+	+	+
KPDR_290 (A1)	0	0	L^{70}→ Q; I^{84}→L; A^{96}→ T; A^{101}→T	+	+	+	+	+	+	+	+

Table 2. *The frequency of amino acid substitutions at various positions in gp41-IDR peptide (Kenya).*

Amino acid and position	Frequency	Amino acid and position	Frequency
W60	0	G83	2
G61	9	I84	19
I62	2	W85	0
K63	0	G86	0
Q64	0	C87	3
L65	0	S88	1
Q66	4	G89	0
A67	4	K90	6
R68	11	L91	14
V69	0	I92	1
L70	3	C93	1
A71	5	T94	17
V72	18	T95	0
E73	0	A96	79
R74	6	V97	0
Y75	1	P98	1
L76	0	W99	0
K77	41	N100	0
D78	1	A101	75
Q79	2	S102	0
Q80	1	W103	0
L81	3		
L82	1	Total substitutions = 331 (151* after removing Con Global Sequence differences)	

There were 331 substitutions in the Consensus gp41-IDR peptides (Kenya) (Table 2). Out of these substitution 151 were due to the substitution in positions A^{96}→N (n = 79) and A^{101}→S (n = 75). Both Consensus gp41-IDR peptides (Kenya) and Consensus gp41-IDR peptides (Global) showed common substitutions rate of 4.2% (n = 151).Most (85%) substitutions occurred at positions G61 (n = 9); R68 (n = 11), V72 (n = 18), K77 (n = 41), I84 (n = 19), L91 (n = 14), T94 (n = 17), A96 (n = 79) and A101 (n = 75). Fifteen (15) amino acid positions (W60, K63, Q64, L65, V69, L76, W85, G86, G89, T95, V97, W99, N100, S102 and W103) were 100% conserved and they did not have any substitution. The most common substitutions as indicated on Table 3 were A^{101}→S (n = 68), A^{96}→ N (n = 50), K^{77}→R (n = 31) and I^{84}→L (n = 17). It is worth noting that the only glycosylation site in the sequence wasN100 that was 100% conserved. Thirty Nine (39) substitutions were single substitutions. The Study identified five cases of deletions {two cases of 60-101(del 96), 60-101(del 63) and 60-101(del 70), 60-101(del 71)} and three cases of insertions{Ins(93a = P), Ins(68a = S, 68b = P), Ins(66a = G, 68a = S), Ins(66a = G, 68a = S)}. Substitutions involving Cysteine Loop were 25 and only one involved substitution of S (S^{88}→A) that is critical for the formation of disulphide links.

Effect of amino acid substitutions on serologic detection: All the 92 HIV positive samples that were sequenced in this study were reactive using the following HIV testing kits:(*HIV* (1 + 2) Antibody (colloidal gold) (KHB Shanghai Kehua Bio-engineering Co, Shanghai, China), First Response™ 1-2.0 (PMC Medical Pty. Ltd, Daman, India), Uni-Gold™ HIV test (Trinity Biotech, USA); Determine™ HIV-1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands), Alere Determine™ HIV-1/2 Ag/Ab Combo (Orgenics Ltd, Yavne, Israel), Aware™ HIV-1/2 BSP (Calypte Biomedical Corporation, Oregon, USA) and Vironostika™ Uni-Form II Ag/Ab ELISA (bioMérieux, Marcy-l'Etoile, France). The same results were obtained when the samples were tested

using the two ELISA systems developed in this study: one using Consensus gp41-IDR peptide (Kenya) and another using Consensus gp41-IDR peptide (Global).The performance of both Consensus gp41-IDR peptides (even after testing all 400 samples in the study was the same.

Table 3. *Various types of amino acid substitutions in gp41-IDR peptide (Kenya) and their frequencies.*

Amino acid and position	Frequency	Amino acid and position	Frequency
A^{101}→S	68	K^{77}→E	1
K^{77}→R	31	D^{78}→H	1
A^{96}→ N	50	Q^{79}→R	1
I^{84}→L	17	S^{88}→A	1
A^{96}→ T	16	K^{90}→V	1
V^{72}→L	15	A^{101}→Y	1
T^{94}→P	12	G^{83}→A	1
A^{96}→T	9	I^{84}→F	1
R^{68}→S	8	R^{99}→W	1
L^{91}→H	7	V^{77}→I	1
V^{69}→I	7	I^{84}→V	1
A^{71}→G	5	C^{87}→S	1
L^{91}→I	10	Q^{66}→K	1
K^{90}→R	5	Q^{66}→H	1
A^{101}→T	5	Q^{66}→R	1
K^{77}→Q	4	R^{74}→S	1
T^{94}→S	4	L^{91}→R	1
K^{77}→V	3	P^{98}→R	1
V^{72}→I	3	A^{101}→V	1
V^{69}→I	7	A^{67}→ T	1
A^{71}→G	5	R^{68}→E	1
K^{90}→R	5	Q^{66}→ P	1
A^{101}→T	5	A^{96}→ S	1
K^{77}→Q	4	R^{74}→A	1
T^{94}→S	4	K^{77}→T	1
K^{77}→V	3	Q^{80}→R	1
V^{72}→I	3	L^{91}→P	1
A^{96}→F	3	L^{70}→Q	1
A^{67}→T	3	G^{83}→R	1
I^{62}→V	2	R^{68}→K	1
L^{70}→ Q	2	Q^{79}→H	1
C^{87}→L	2	C^{93}→S	1
R^{74}→K	2	L^{81}→P	1
R^{74}→G	2	L^{82}→P	1
L^{81}→I	2	K^{77}→G	1
		V^{70}→L	1
		Y^{75}→F	1
		T^{94}→A	1
		V^{69}→M	1

4. Discussions

This study established that there was no significance difference between the performance of the HIV ELISA Testing kit that was developed with Consensus gp41-IDR peptide (Kenya) and that which was developed using Consensus gp41-IDR peptide (Global). The study also established that the HIV testing kits that are used in Kenya are able to detect all HIV infections irrespective of numerous amino acid substitutions in the Immunodominant Region of p41. This study is in agreement a study by Masciotra *et al.* (2000) who established that consensus gp41-IDR from group M peptides (WGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTA VPWNASW) was able to detect all 130 group M sera (10 subtype A, 21 subtype B, 13 subtype B9, 20 subtype C, 21 subtype D, 14 subtype E, 25 subtype F, and 6 subtype G). It also agrees with the findings by Bártolo and Taveira (2012) that despite the high genetic divergence between HIV-1 groups M and N, all group N infections had been detected using five commercial HIV immunoassays. Lihana *et al* (2009) found the prevalence of HIV subtype A1 to be predominant in Nairobi at 65% as compared to the prevalence of 85% established by this study. Several studies have been done to improve HIV-1 serologic detection by concentrating on the inclusion of recombinant and/or synthetic peptides representing sequencing of IDRs within the gp41 region of various subtypes or recombinants of HIV (7). One such study designed a multisubtype gp41 recombinant antigen (rIDR-M) with incorporation of three variant sequences. The first sequence was most common among subtypes A, B, C, F, G, H, J, and K and recombinants AG, AB, AC, BF, andBG.37. The second sequence was most common only in subtype AE from Thailand, and the third sequence was most common among subtypes D and recombinant AD (7). It worth noting that the all the kits used in this study were able to detect HIV sub-type A, C and D.Contrary to the studies by Dorn *et al* (2000) who found that the CxxxxxC in which the Cysteine loop was highly conserved, this study substitutions fairly high level of substitutions of 7.85% (n=26) of 14 were at position L91. Substitutions at the location especially that of L^{91}→H (n =7) has been known to reduce the activity of the antibodies that target gp41-IDR peptides (13). This study however found detections of samples displaying this substitution in agreement with similar results that were found by Dorn *et al* (2000) which was stipulated to be due to detections by antibodies that target other epitopes. Johnson *et al* (1992) found that K77→R substitutions could reduce CTL recognition possibly through the interference of peptide-major histocompatibility complex interactions. In the prevalence of K77→R substitutions was found to be 23.3% and further studies may be required to be done to establish how this could affect the CTL recognition in Kenya.

This study concludes that although the HIV Consensus gp41-IDR peptides (Kenya) showed marked substitutions in respect to Consensus gp41-IDR peptide (Global) there was no difference in effects on the performance of the HIV-1 Antibody-Based Detection Kits in use in Kenya.

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