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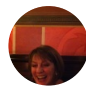

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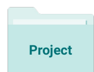

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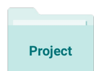

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Running Title

Primary HIV Phenotypic Resistance

Key words

HIV-1 subtype; Phenotypic susceptibility; Antiretroviral naïve; Brazil

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Abstract

To evaluate antiretroviral phenotypic susceptibility of wild-type HIV-1 strains circulating in Brazil, samples from antiretroviral-naïve individuals infected with subtypes C (n=16), F (n=9), or B/F (n=7), where reverse transcriptase is B and protease is F, were phenotyped using the Antivirogram^{TM} Assay (Virco, Mechelen, Belgium). Reduced susceptibility to protease inhibitors (PIs) was observed in one C and three F isolates. None of these samples had any known PI resistance mutations. The Phenotypic Fold Change to one PI was above the biologic cut-off in 3 of 96 (3.1%) of clade F phenotypic determinations and 1 of 96 (1.0%) of C. Phenotypic resistance to at least one nucleoside reverse transcriptase inhibitor (NRTI) was found for two B/F, four C, and three F isolates. The Phenotypic Fold Change in susceptibility to NRTIs was above the cut-off value in 9 of 111 (8.1%) of clade C determinations, as compared to 3 of 63 (4.8%) for clade F and 2 of 49 (4.1%) for clade B. The Phenotypic Fold Change to non-NRTI (NNRTI) was above the cut-off in 7 of 32 (21.9%) of C isolates determinations, whereas none of the F isolates had decrease of susceptibility. Only two of the sixteen C samples had a known NNRTI resistance mutation. The NNRTI Fold Change was above the cut-off value in 3 of 14 (21.4%) of phenotypic determination of Brazilian B/F recombinants, representing clade B reverse transcriptase. NNRTI susceptibility should be better investigated in clade C and B/F recombinants.

Word count = 241 (250 maximum)

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Introduction

Drug resistance testing has become an important tool in the management of HIV-infected individuals undergoing antiretroviral therapy.[1] Genotyping and phenotyping methods appear to be equally useful for determining the susceptibility of HIV-1 to antiretroviral drugs.[2] Genotyping identifies mutations in the HIV genome associated with antiretroviral resistance, whereas phenotyping examines the relative susceptibility of viruses to different antiretroviral concentrations *in vitro*. It is conceivable that the genetic diversity of HIV-1 influences the susceptibility to antiretroviral drugs. Drug susceptibility tests have been designed for subtype B strains and are performed mainly on those strains. However, the increasing global prevalence of non-B subtypes creates the need to determine the performance of commercial drug resistance assays in testing these HIV-1 subtypes. Furthermore, it has been demonstrated that specific mutations or pathways of antiretroviral resistance differ between HIV-1 clades.[34] Some retrospective analysis suggests that individuals infected with clade F HIV-1 respond more poorly to antiretroviral therapy than clade B-infected individuals.[5] It has also been suggested that the *L89M* polymorphism, which is highly prevalent in the clade F protease[6], plays a role in the antiretroviral response because this polymorphism decreases the susceptibility of viruses to most protease inhibitors (PIs).[7] In contrast, it has been also speculated that the *I93L* polymorphism increases the susceptibility of clade C samples to Lopinavir.[8] Although the *I93L* polymorphism is also common in clade F viruses, the effect in the F subtype has not been determined. In Brazil, the HIV-1 clades B, C, and F co-circulate, and B/F recombinants account for a significant proportion of all circulating clades.[9, 10] In this study, we evaluated the mutation profile and *in vitro* antiretroviral response of samples from antiretroviral-naïve Brazilian patients infected with subtypes C, F, or B/F. We evaluated the phenotypic susceptibility and genotypic correlates of resistance in antiretroviral-naïve strains to assess the natural susceptibility of the C and F HIV subtypes to PIs as well as nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs).

Materials and Methods

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Isolates from 32 antiretroviral-naïve Brazilian patients were evaluated. There were 16 subtype C, 9 subtype F1, and 7 subtype B/F recombinant isolates. All B/F recombinant samples carried a F1 protease and a B reverse transcriptase. Viruses from plasma samples were genotyped at the Federal Universities of Sao Paulo and Rio de Janeiro, Brazil, using the ViroSeq System (Celera Diagnostics, Alameda, CA, USA). Sequences were analyzed using an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). All genotyping analysis was conducted on a portion of the *pol* gene that spans the reverse transcriptase and protease regions. Samples identified as recombinants were analyzed using SimPlot version 3.5.1[11] and confirmed by bootscanning analysis. The basic principle of bootscanning is that mosaicism is suggested by high levels of phylogenetic relatedness between a query sequence and more than one subtype reference sequence in different genomic regions.[12] Sample sets were subjected to antiretroviral phenotypic analysis using the Antivirogram™ Assay (Virco, Mechelen, Belgium). This phenotyping assay uses HIV-1 genomes generated through the recombination of PCR amplified *pol* products derived from patient viruses and a subtype B proviral clone with a deletion of the protease and reverse transcriptase regions. Recombinant viral production and detection methods were conducted as previously described.[13, 14] For clade C, F and B/F recombinant isolates obtained from treatment-naïve subjects, the mean, median, and interquartile range of Fold Change values for each drug, as determined using the Antivirogram™ assay, were compared to the same parameters for clade B isolates in the Virco database. The mutation patterns associated with reduced susceptibility to one or more drugs in clade C, F, and B/F isolates were evaluated and compared to the genotypic correlates of drug resistance described for clade B isolates.

Results

The following are shown in Tables 1A and 1B: sample identification; protease and reverse transcriptase subtypes; polymorphisms/mutations at codons associated with resistance; Phenotypic Fold Change in susceptibility to PIs, NRTIs and NNRTIs; and mean Fold Change by subtype.[15]

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The Antivirogram^{TM} HIV-1 Fold Change values are interpreted using cut-off values to separate viral isolates with high antiretroviral susceptibility from those with low antiretroviral susceptibility. Biological cut-off values are derived from *in vitro* experiments and mark the upper limit of natural variation in phenotypic susceptibility among wild-type viruses, which includes 97.5% of strains.[15] Although these biological cut-off values were typically calculated using clade B strains, we analyzed the percentage of viruses from clades C, F and Brazilian B/F recombinant that remained above the biological cut-off value. There was in total 96 phenotypic determinations for PIs among the clade 16 subtype C isolates as well as 96 phenotypic determinations for PIs the 16 strains with protease F, 9 of which harbored subtype F RT and 7 harboring subtype B RT. For PI susceptibility, 1 out of 96 phenotypic determinations (1.0%) of the isolates from clade C and three out of 96 determinations (3.1%) from clade F had Fold Change values above the upper limit of the biological cut-off values (Fischer exact test of 0.3). For NRTI susceptibility, nine out of 111 phenotypic determinations (8.1%) of Fold Change values were above the biological cut-off for clade C isolates, versus three out of 63 determinations (4.8%) and two out of 49 determinations (4.1%) for clades F and B/F, respectively (Fischer exact test between groups non-significant). The phenotyping results for NNRTI susceptibility showed that seven out of 32 phenotypic determinations (21.9%) revealed Fold Change values above the biological cut-off value in clade C isolates. None of the clade F isolates showed decreased susceptibility to NNRTIs (Fischer exact test comparing clade C versus F p=0.03). Most importantly, clade C samples did not have any mutation known to correlate with NRTI resistance, and only two samples had mutations correlating with NNRTI resistance (mutations A98G and K103N in samples BR76 and BR87, respectively, Table 1B). Surprisingly, three out of 14 determinations (21.4%) of the Brazilian clade B/F isolates had Fold Changes above the biological cut-off value for NNRTI susceptibility, although no statistical differences were detected for NNRTI resistance between clades B/F versus F (Fischer exact tests, p=0;07) or B/F versus C (p=0.6).

All but four viral samples were fully susceptible to all six PIs tested (Table 1A). Three subtype F samples (BR38, BR43, and BR44) showed reduced susceptibility to PIs. BR38 had a Fold Change of 1.8 for Saquinavir and carried the following protease polymorphisms/mutations: V3I, I15V, E35D, M36I, S37N, R41K, R57K, D60E, Q61E, L63P, K70T, and V77I. The BR43 isolate also showed decreased susceptibility to

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Saquinavir (Fold Change of 2.1) and carried the protease polymorphisms/mutations V3I, L10V, I13V, I15V, G16E, K20M, E35D, M36I, S37N, R41K, R57K, Y59H, Q61N, I72T, and L89M. The BR44 isolate had a Fold Change of 2.3 for Lopinavir and contained the amino acid substitutions V3I, I15V, G17E, K20R, E35D, M36I, S37N, R57K, Q61N, L63T, E65D, and I72V. Only one clade C isolate (BR86) had a low level of resistance, with a 0.1 Fold Change above the biological cut-off value. This isolate contained the following amino acid substitutions: V3I, I15V, M36I, S37K/N, R41N, L63I/P/S/T, H69K, V82I/V, L89M, and I93L. As shown in Table 1A, these four viruses had Fold Changes discreetly above the biological cut-off values for each respective drug.[15] Since other strains tested here displayed the same mutation profile without a reduced PI susceptibility, direct involvement of those amino acid substitutions with drug resistance could not be speculated. One exception was the Y59H substitution. This substitution was only detected in the BR43 isolate, which showed a loss of susceptibility to Saquinavir. All twenty-eight of the remaining isolates (13 of subtype F and 15 of subtype C) were fully susceptible to all PIs tested. The mean Phenotypic Fold Change per subtype sample set was nearly 1.0, which is comparable to that of subtype B wild-type strains.

Accessory amino acid substitutions that correlated with selective pressure from antiretroviral drugs among subtype B, including substitutions at codons 10, 20, 36, 63, 77, and 82, were detected in the protease region of non-B samples. Changes were found at the following prevalences: L10I (12.5% in subtype F, 6.2% in subtype C); L10V (18.7% in subtype F, 6.2% in subtype C); K20R (43.7% in subtype F, 12.5% in subtype C); M36I (87.5% in subtype F, 81.2 in subtype C); M36T (12.5% in subtype C); L63P (12.5% in subtype F and subtype C); L63A/G/H/I/L/Q/S/T/V (31.2% in subtype F, 43.7% in subtype C); V77I (18.7% in subtype F) and V82I (6.2% in subtype F, 31.2% in subtype C). The L89M polymorphism did not have any effect on PI susceptibility among the clade F isolates (Fold Change ≤ 1.0). The I93L polymorphism also did not have any effect on PI susceptibility among clade C isolates, as shown by the mean Phenotypic Fold Change of 1.0 in susceptibility to Lopinavir. Although previous studies have shown that the L89M polymorphism has an impact on Saquinavir, Nelfinavir, Amprenavir and Lopinavir susceptibility,[7, 16] we found no data to support a consistent change in PI susceptibility related to this polymorphism. It has also previously been reported that susceptibility to Lopinavir increases with the I93L substitution in clade C isolates, and the poor response to

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PI therapy is empowered by I93L.[8, 17] In contrast, we found I93L in all subtype C samples analyzed. The mean Fold Change value for the clade C samples was very close to 1.0 (0.8), and the susceptibility to other PIs was not compromised. The remaining polymorphisms are of uncertain significance. As previously described,[18] 100% of the clade F isolates carried V3I and R57K; 81.2% carried S37N, R41K, and Q61N; 62.5% carried E35D; 56.2% carried I15V; and 50% carried L89M. For clade C, 100% of the isolates carried S37K, R41N, H69K, I89M, and I93L; 87.5% carried I15V; and 31.2% carried G16A/E. Notably, subtype F isolates had S37N, R41K, and R57K polymorphisms, whereas subtype C isolates had S37K, R41N, and H69K polymorphisms in all samples. When different substitutions related to PI resistance in clade B (K20I, M36V/L, and L63S/A/T/F) were considered, all but two subtype F isolates (87.5%) carried two to four mutations. Nearly 62% of subtype C samples carried one to three of those mutations.

We also analyzed the susceptibility of clades C and F to reverse transcriptase inhibitors (seven NRTIs and two NNRTIs). The number of Brazilian isolates included from each clade was as follows: 7 subtype B/F, 16 subtype C and 9 subtype F (Table 1B). Decreased susceptibility to at least one reverse transcriptase inhibitors was observed in 10 viral isolates. There were 2 clade B/F (BR36 and BR39), 3 clade F (BR43, BR44 and BR46) and 6 clade C (BR76, BR82, BR86, BR87, BR94, and BR99) samples with decreased susceptibility to at least one reverse transcriptase inhibitor (Table 1B). Because clades C and F carried many reverse transcriptase polymorphisms, Table 1B shows only the amino acid substitutions known to be related to drug resistance.

Within the clade F samples, BR43 had decreased susceptibility to ddI with a Fold Change of 0.1 above the cut-off value. BR44 and BR46 had decreased susceptibility to Zidovudine with Fold Changes of 0.2 and 0.5 above the cut-off values, respectively. These three clade F samples showed loss of susceptibility without any amino acid substitutions previously described as related to antiretroviral resistance.

Within the clade C samples, BR76 had the A98G mutation and a Phenotypic Fold Change of 20.7 to Nevirapine, whereas BR87 had the K103N mutation and Phenotypic Fold Changes of 18.7 and 61.6 to Efavirenz and Nevirapine, respectively. The BR86 isolate had a decreased susceptibility to Abacavir, Zidovudine and Efavirenz but only one NRTI resistance-related codon that was previously described. However, this codon had a different amino acid substitution: E40D. BR43 of clade F also had this amino acid change,

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but no decreased susceptibility was observed. The other four subtype C isolates with decreased susceptibility to NNRTIs had no known resistance mutations. The mean Fold Change values of clade F samples were comparable to those of clade B for all reverse transcriptase inhibitors tested. The mean Fold Change values were also near 1.0 for the clade C samples, with the exception of susceptibility to Efavirenz (2.6) and Nevirapine (7.1).

As seen in Table 1b, two out of seven BFs samples presented Fold Change above Biological cut-offs for NRTIs (ZDV and FTC); compared to three out of nine F samples (two samples to ZDV and one to ddI), and four out of 16 subtype C samples (ZDV three samples, d4T two samples and ddI, abacavir and tenofovir one sample each). The study that determined the Biological cut-offs for this Phenotypic assay evaluated 1650 samples for ZDV, [15]and considering the 97.5th percentile profile for Fold Change, it was detected that prevalence of samples above Biological cut-offs were higher in our set of F subtype strains (Fischer´s exact test p=0.02), and C strains (Fischer´s exact test p=0.007). For NRTIs, no other differences were statistically different, with a trend for d4T and clade C, where 1638 samples have been originally tested for this drug by Verlinder et al (Fischer´s exact test p=0.06). [15]

Two out of seven BF samples presented Fold Change above Biological cut-offs to NNRTIs (two to efavirenz and one to neveripine), compared to zero F samples and six C samples (five to nevirapina and two to efavirenz). Again here, compared to the original study that defined the Biological cut-offs which evaluated 2333 samples from naïve individual to efavirenz and according to the 97.5th percentile profile, prevalence of samples with Fold Changes above Biological cut-off among our BF set of samples were higher (Fischer´s exact test p=0.01). Also, when we excluded the two samples that harbored transmitted resistant mutations to NNRTIs in the set of subtype C samples (samples BR76 and BR87, Table 1B), and compared the prevalence of Fold Change above Biological cut-off to nevirapina with the 2357 samples investigated by Verlinden et al, the prevalence was higher in our set of samples (Fischer´s exact test p=0.007). We also found three F samples with Fold Change above to Biological cut-offs to PIs, two to saquinavir, and one to lopinavir, and one subtype C sample to saquinavir (Table 1a).

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Discussion

Although genetic polymorphisms at positions associated with PI resistance were found at a higher frequency in the two non-B subtypes isolated from antiretroviral-naïve subjects, no phenotypic decrease in PI susceptibility was identified in this sample set. However, a decrease in susceptibility to NRTIs and NNRTIs was found among the non-B HIV-1 samples. Remarkably, only two of the eleven samples with decreased susceptibility to antiretrovirals revealed known resistance mutations related to reverse transcriptase inhibitors (A98G and K103N), suggesting that the genotypic correlates of decreased susceptibility among clade C, F and B/F recombinants is not completely understood. Of note, the A98G substitution is related to the decrease in susceptibility to etravirine.[19] One recent study demonstrated that the rate of resistance-related mutation acquisition in clade C samples may be inferior to that of clade B samples.[20] One reasonable explanation for the lower prevalence of mutations in clade C-infected individuals than in clade B-infected individuals is that some correlates of genotypic resistance in HIV-1 clade C may be unknown.

Another hypothetical explanation for the lack of mutations known to confer resistance to reverse transcriptase inhibitors is that genetic mutations outside of the reverse transcriptase catalytic region may influence susceptibility to certain reverse transcriptase inhibitors. In fact, there is mounting data that mutations in the RT connection and RNase H regions lead to NRTI or NNRTI resistance. [21, 22] Resistance mutations outside of the reverse transcriptase region may explain the lack of resistance mutations found because B/F recombinant samples cluster with clade B in the reverse transcriptase region. However, one B/F recombinant sample showed resistance to NRTIs and two samples had resistance to NNRTIs. Sequencing outside of the protease and reverse transcriptase catalytic regions was not performed; therefore, we are unable to assign the outside regions to any particular HIV-1 subtype.

Although all clade C samples tested had the I93L polymorphism in the protease region, we were unable to confirm previous results that suggested *in vitro* hypersusceptibility to Lopinavir because of this natural polymorphism.[8] Additionally, the I93L substitution has been related to lower *in vivo* susceptibility to Indinavir and Nelfinavir among clade B-infected individuals[23], which also was not confirmed in the present study. We were also

unable to confirm that the L89M mutation in clade F samples would compromise the natural susceptibility to PIs, as previously described. [7] However, it can be argued that the high number of clade-related polymorphisms found in non-B strains acts in decreasing the genetic barrier to PI resistance, even in the absence of baseline resistance to PIs, as described in the present study. It has been reported that the polymorphisms found at positions 10 and 36 are the strongest predictors of therapeutic failure, appearing in nearly 40% of antiretroviral-naive subjects who failed PI-based therapy.[24] Notably, the M36I mutation was present in 87.5% of the F- and 75.0% of the C-subtype proteases in our study, and that the phenotypic assays did not recognize any significant loss in PI susceptibility.

Although previously antiretroviral-naive individuals treated with boosted PIs do not typically have PI mutations or virologic resistance, [25-28] one study revealed a genetic progression in five patients treated with boosted Lopinavir monotherapy, two infected with clade B HIV-1 and three infected with CRF02_AG.[29] Although no phenotypic resistance was confirmed in these two clade B isolates, it has been confirmed in two CRF02_AG strains with the emergence of L76V and other mutations. In those cases, it can be speculated that the high number of polymorphisms in non-B strains allows genetic evolution and selective pressure of PIs.

Another point of great interest is the relationship between the genetic diversity of HIV and response to treatment. An unrecognized risk of antiretroviral failure may be related to a high number of natural polymorphisms in non-B strains. Although the results of some studies indicate that the level of response is lower among patients infected with non-B HIV, [5] other studies have shown the opposite. [30]

We recognize that the small size of the sample in this study is a major limitation and that further studies are needed to confirm our results comparing Non-B to B strains. Nevertheless, we propose that until better correlates of genotypic resistance are available, phenotypic tests for non-B clades should be more widely accessible.

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Author Disclosure

PM is a current employee of LifeTechnologies, Brazil; RD has served on advisory boards and spoken at symposia or local events for Virco; MCS, JS, AS, LJ, MS and RD have no

conflict of interest. Virco had not played any decision-making role in the design of this study.

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Funding

This work was partially supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (04/15856-9). Antivirogram^{TM} assays were supported and executed by Virco (Mechelen, Belgium).

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TABLE 1A. Results of the phenotypic susceptibility of HIV-1 Brazilian clade F and C samples to protease inhibitors (PIs) using the Antivirogram™ Assay and protease genotypic profile.

Sample ID	Protease Subtype	FC in PI susceptibility vs. wild-type: APV	ATV	IDV	LPV	NFV	SQV	Protease polymorphisms/mutations
		*Virco biological cut-off value**						
		(1.8)	(2.0)	(2.1)	(1.6)	(2.3)	(1.7)	
BR29	F	1.1	0.7	0.5	1.6	1.4	0.8	V3I R41K R57K Q61N K70R L89M
BR35	F	1	1	0.7	0.6	0.8	0.4	V3I L10I I13V L33V E35D M36I S37N R41K R57K D60E Q61N G68E L89M
BR36	F	0.7	1.1	1	1.6	0.7	1.5	V3I G17D K20R E35D M36I S37N R41K K43R R57K Q61N I72R
BR37	F	0.8	1.1	1	0.6	0.9	0.5	V3I T12I I13V M36I S37N R41K R57K Q61H/N L89M
BR38	F	1.4	0.9	0.7	1.2	1.7	**1.8**	V3I I15V E35D M36I S37N R41K R57K D60E Q61E L63P K70T V77I
BR39	F	0.3	0.2	1.4	0.7	0.6	0.3	V3I T12K I15V L19I E35D M36I S37N R41K R57K Q61N E65D I72T
BR40	F	0.6	0.5	0.9	0.9	1	1	V3I L10I I13V G17E K20R M36I S37E R57K Q61N L63AN K70R
BR31	F	0.9	1	0.8	0.6	0.8	0.5	V3I I13I/V I15I/L M36I S37N R41K R57K Q61N H69Y L89M
BR43	F	1.2	1.3	0.7	1.1	1.2	**2.1**	V3I L10I I13V I15V G16E K20M E35D M36I S37N R41K R57K Y59H Q61N I72R L89M
BR44	F	0.6	0.4	0.6	**2.3**	1.4	0.6	V3I I15V G17E K20R E35D M36I S37N R57K Q61N L63T E65D I72V
BR46	F	1.1	1.5	0.5	1.3	1.4	0.9	V3I I15V K20R E35D M36I S37N P39Q R41K R57K D60E Q61D L63P/S L89M
BR48	F	0.3	0.1	0.2	0.7	1.1	0.2	V3I L10V I15V S37E R57K Q61N L63A I64L I72V V77I I93L
BR50	F	1.3	0.7	0.7	1.1	0.5	0.5	V3I L10I T12S I13V K14R G16E K20R E35D M36I S37N R41K R57K Q61N L63V I72T
BR51	F	0.3	0.6	1.5	0.9	1	0.9	V3I I15V E35D M36I S37N P39Q R41K R57K Q61N V77I L89M
BR52	F	0.3	0.4	0.3	0.5	0.3	0.4	V3I I15V K20R M36I/L S37K R41N H69K V82I L89M I93L
BR53	F	0.4	0.3	0.2	0.7	0.8	0.3	V3I I15V E35D M36I S37N R41K R57K Q61N L63S E65D I72T
Mean		**0.8**	**0.7**	**0.7**	**1**	**0.9**	**0.8**	
BR76	C	0.2	0.5	0.3	0.7	0.4	0.5	V3I I15V G16A M36I S37K R41N H69K L89M I93L
BR78	C	0.2	0.6	0.3	0.6	0.4	0.5	V3I I15V G16E M36I S37K R41N H69K L89M I93L
BR79	C	0.6	0.8	0.5	0.9	1.4	0.5	V3I I15V M36I S37K R41N L63T H69K L89M I93L
BR80	C	0.7	0.8	0.4	0.6	0.4	0.9	V3I I15V M36I S37K R41N L63T H69K V82I L89M I93L
BR81	C	0.2	0.1	0.2	0.7	0.7	0	V3I I15V M36I S37K R41N L63GN H69K L89M I93L
BR82	C	0.7	0.6	0.2	1.2	1	0.6	V3I G16E M36I S37K R41N L63H/U/Q/V I64L H69K L89M I93L
BR83	C	0.5	0.6	0.8	1	1.1	0.9	V3I I15V L19I M36I S37K R41N M46V H69K V82I L89M I93L
BR84	C	0.4	0.9	0.8	0.7	0.5	0.7	V3I T12P I15V M36T S37K R41N L63T H69K L89M I93L
BR86	C	1.3	1.1	0.8	1.2	1.6	**1.8**	V3I I15V M36I S37K/N R41N L63I/P/S/T H69K V82IN L89M I93L
BR87	C	0.9	1	1.8	0.6	0.5	1.4	V3I I15V L19I S37K R41I D60E H69K L89M I93L
BR88	C	0.3	0.3	0.2	0.8	1.3	0.8	V3I T12N I15V M36I S37N R41K H69K L89M I93L
BR89	C	0.5	0.6	0.6	0.7	1.1	0.7	V3I L10I I13V K14R M36I/T S37K R41N L63P H69K L89M I93L
BR91	C	0.8	1.1	0.3	1	1.3	0.5	V3I I15V G16E K20R E35D M36I S37K P39S R41N/S H69K L89M I93L
BR92	C	0.2	0.4	0.2	0.3	0.2	0.3	V3I T12K K14R I15V M36I S37K R41N H69K V82I L89M I93L
BR94	C	0.6	0.4	0.3	0.6	0.6	0.5	V3I I15V S37K R41N L63V H69K V82I L89M I93L
BR99	C	0.8	1.2	1.7	1.4	1.2	0.7	V3I K14R I15V G16E K20R M36I S37K P39S R41N H69K L89M I93L
Mean		**0.6**	**0.7**	**0.6**	**0.8**	**0.9**	**0.7**	

FC, fold change; PI, protease inhibitor; APV, Amprenavir; AZT, Zidovudine; IDV, Indinavir; LPV, Lopinavir; NFV, Nelfinavir; SQV, Saquinavir. Mean fold changes and fold changes above biological cutoff are in bold.

*Values refer to the second generation Virco biological cut-off values.[13]

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TABLE 1B. Results of phenotypic susceptibility of HIV-1 Brazilian samples to reverse transcriptase inhibitors (NRTIs and NNRTIs) using Antivirogram™ Assay and reverse

		FC susceptibility vs. wild-type									
		NRTIs							NNRTIs		RT resistance mutation codons
		*Virco biological cut-off value**							*Virco biological cut-off*		
Sample ID	RT Subtype	ABC (2.2)	ddI (2.2)	FTC (3.5)	3TC (2.4)	d4T (2.3)	TDF (2.1)	AZT (2.1)	EFV (3.4)	NVP (5.5)	
BR29	B/F	0.7	0.5	0.4	0.8	0.4	1	**2.7**	1.6	0.7	
BR35	B/F	0.5	0.7	2	0.7	0.6	0.4	1.2	0.9	2.8	
BR36	B/F	0.5	1.1	1.2	0.7	1.6	1	0.7	**4.1**	**7.2**	
BR37	B/F	1.1	1	0.7	1.1	1.1	1	1.2	2.6	1.2	
BR38	B/F	0.7	1.9	0.9	1.4	1.8	1.7	0.7	1	1.1	
BR39	B/F	0.8	2.1	**3.6**	0.6	0.4	0.7	0.7	**3.8**	1.6	
BR40	B/F	0.5	0.6	1.1	1.1	0.8	0.4	1.9	0.2	0.9	
Mean		**0.7**	**1.1**	**1.4**	**0.9**	**1,0**	**0.9**	**1.3**	**2,0**	**2.2**	
BR31	F	0.8	0.9	0.7	0.5	1.3	0.8	1.2	1.5	1.7	
BR43	F	0.2	**2.3**	0.4	1.1	1.5	1	0.6	0.7	1.1	**E40D**
BR44	F	1.7	0.7	1.3	0.6	1.5	0.6	**2.9**	1.2	0.8	
BR46	F	0.8	1.1	1	0.7	2.2	0.5	**3.2**	0.7	1.8	
BR48	F	0.2	0.2	0.1	0.4	0.2	0.6	0.2	0.5	0.5	**150V**
BR50	F	1	0.7	1.4	0.9	1.3	1.4	1.2	1.1	0.9	
BR51	F	1.6	0.3	0.5	0.6	0.3	0.3	0.3	0.2	0.7	**G333D**
BR52	F	0.3	0.5	0.9	1.1	0.7	0.4	1.8	0.9	0.2	
BR53	F	0.5	0.2	0.3	0.6	0.2	0.5	0.4	0.2	0.2	
Mean		**0.7**	**0.9**	**1.1**	**0.8**	**1,0**	**0.8**	**1.3**	**1.4**	**1.5**	
BR76	C	0.4	0.5	0.7	1	0.3	1.4	0.8	1.9	**20.7**	**A98G**
BR78	C	0.4	0.9	1	0.9	0.4	0.9	0.6	0.2	1.2	
BR79	C	0.4	0.8	1.7	1.2	0.5	1.4	0.5	2.2	0.8	
BR80	C	0.6	0.4	3.3	0.6	0.4	0.8	1.9	2.1	4.7	**A98S**
BR81	C	0.2	0.2	0.1	0.7	0.2	0.6	0.2	0.5	0.5	
BR82	C	0.6	1.8	1.5	1.6	**2.6**	1	1.1	2	**6.3**	
BR83	C	0.5	1.2	1.3	0.8	0.6	1.4	0.9	2.2	0.9	
BR84	C	0.4	1.3	0.5	1.6	0.3	0.8	0.6	2.3	0.4	
BR86	C	**2.7**		1.4	2	2	1.5	**3.4**	**3.9**	0.8	**E40D**
BR87	C	1.9	1.8	2.2	1.1	2.1	1.4	**2.6**	**18.7**	**61.6**	**K103N**
BR88	C	0.2	0.3	0.3	0.7	0.3	1.6	0.5	0.5	0.7	
BR89	C	0.8	0.5	1.4	0.8	0.4	0.6	0.9	0.6	0.7	
BR91	C	1.3	1	0.9	1.2	1	0.5	1.2	0.9	0.5	
BR92	C	0.3	0.3	0.6	0.8	0.3	0.2	0.7	0.5	0.3	
BR94	C	0.5	0.7	0.1	0.6	0.6	0.8	0.4	0.5	**6.1**	
BR99	C	1.2	**2.4**	1.1	**2.6**	**4**	**2.4**	**2.3**	3	**7.2**	
Mean		**0.8**	**0.9**	**1.1**	**1.1**	**1,0**	**1.1**	**1.2**	**2.6**	**7.1**	

FC, fold change; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; RT, reverse transcriptase; ABC, Abacavir; ddI, Didanosine; FTC, Entricitabine; 3TC, Lamivudine; d4T, Stavudine; TDF, Tenofovir; AZT, Zidovudine; EFV, Efavirenz; NVP, Nevirapine. Mean fold changes and fold changes above biological cutoff are in bold.

*Values refer to the second generation Virco biological cut-off values.[13]

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