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Comparison of Genotypic and Virtual Phenotypic Drug Resistance Interpretations with Laboratory-based Phenotypes among CRF01_AE and Subtype B HIV-infected individuals

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Abstract

Objectives—HIV drug resistance assessments and interpretations can be obtained from genotyping (GT), virtual phenotyping (VP) and laboratory-based phenotyping (PT). We compared resistance calls obtained from GT and VP with those from PT (GT-PT and VP-PT) among CRF01_AE and subtype B HIV-1 infected patients.

Methods—GT predictions were obtained from the Stanford HIV database. VP and PT were obtained from Janssen Diagnostics BVBA's *vircoType*TM HIV-1 and Antivirogram®, respectively. With PT assumed as the “gold standard”, the area under the curve (AUC) and the Bland-Altman plot were used to assess the level of agreement in resistance interpretations.

Results—A total of 80 CRF01_AE samples from Asia and 100 subtype B from Janssen Diagnostics BVBA's database were analysed. CRF01_AE showed discordances ranging from 3 to 27 samples for GT-PT and 1 to 20 samples for VP-PT. The GT-PT and VP-PT AUCs were 0.76 to 0.97 and 0.81 to 0.99, respectively. Subtype B showed 3 to 61 discordances for GT-PT and 2 to 75 discordances for VP-PT. The AUCs ranged from 0.55 to 0.95 for GT-PT and 0.55 to 0.97 for VP-

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Genbank Accession Numbers

The accession numbers for the sequences used in this analysis are: KP877129 - KP877308.

Conflicts of interest

PVDE is a full-time employee of Janssen Diagnostics BVBA, though no commercial interest is affiliated to the services provided. All other authors declared no conflicts of interest.

PT. Didanosine had the highest proportion of discordances and/or AUC in all comparisons. The patient with the largest didanosine FC difference in each subtype harboured Q151M mutation. Overall, GT and VP predictions for CRF01_AE performed significantly better than subtype B for three NRTIs.

Conclusions—Although discrepancies exist, GT and VP resistance interpretations in HIV-1 CRF01_AE strains were highly robust in comparison with the gold-standard PT.

Keywords

drug resistance; algorithm; subtype

Introduction

HIV drug resistance testing plays an important role in the selection of appropriate treatment regimens for HIV infected individuals. Several methods based on genotypic testing (GT) and/or phenotypic testing (PT) are currently available in clinical practice, however the assessment of HIV-1 resistance usually relies on GT as phenotypic assays are often more complex and costly [Tang and Shafer, 2012].

GT works by amplifying and sequencing the HIV-1 *pol* gene, in which the resulting resistance-associated mutations (RAMs) can be analysed to determine the virus' susceptibility to antiretroviral drugs (ARVs). PT involves laboratory procedures that directly measures the virus' response to each drug being evaluated. That is, PT is a measure of the amount of drug required to inhibit 50% of viral replication (IC_{50}) relative to a drug-susceptible reference control strain (wild-type). It is usually reported as the fold change (FC) in susceptibility, which is the ratio of the IC_{50} for the drug being tested for the patient's virus over the IC_{50} of the wild-type virus. Another method which combines both genotypic and phenotypic information to predict drug susceptibility is virtual phenotyping (VP). VP predicts ARV susceptibility by comparing mutations obtained from GT with a database containing both genotypic and phenotypic information. The VP FC value is then calculated from a mathematical equation, which can then be used to interpret varying degrees of ARV resistance [Vermeiren et al., 2007].

Drug resistance predictions from GT and VP can be obtained via software-based systems. The publicly available Stanford University HIV Drug Resistance Database [Liu and Shafer, 2006] (Stanford HIVdb) predicts genotypic drug resistance based on a pre-determined rules based system utilising the consensus HIV-1 subtype B sequence [Rhee et al., 2003; Shafer, 2006]. Janssen Diagnostics BVBA's (Beerse, Belgium) proprietary software, vircoTypeTM HIV-1, provided VP through linear regression modelling based on a consensus HXB2 strain and biological- and/or clinical cut offs [Pattery et al., 2012; Steegen et al., 2010].

As HIV-1 subtype B is commonly used as the consensus sequence, there are concerns regarding the accuracy of these drug resistance interpretation systems when analysing HIV-1 non-B subtypes. Between 2004–2007, subtype B only accounted for 11% of global infections and was predominant in countries including North America, the Caribbean, Latin America, western and central Europe and Australia. HIV-1 AE circulating recombinant form

(CRF01_AE) was predominant in south and south-east Asia and subtype C in southern Africa, Ethiopia and India[Hemelaar et al., 2011]. Previous studies have shown varying degree of discrepancies between different interpretation systems in the prediction of drug resistance in non-B subtypes[Jiamsakul et al., 2012; Vergne et al., 2006; Yebra et al., 2010]. It is recognised that more sequences from non-B subtypes are needed to improve the robustness of the reference databases utilised to derive predicted resistance calls. Further comparison of genotypes and virtual phenotypes to laboratory-performed phenotypic resistance profiles would increase the validity of these resistance interpretation tools for non-B subtypes. Additionally, comparing the level of accuracy between sequences from non-B HIV-1 infected individuals from resource-limited settings and subtype B sequences from resource-rich countries will further help improve the awareness of the differences in the performance of resistance algorithms for these two subtypes.

The objectives of this study were to examine the agreement in drug resistance predictions by comparing results obtained from GT(Stanford HIVdb) and VP (vircoType™ HIV-1) with PT (Antivirogram®[Pattery et al., 2012]) in CRF01_AE and subtype B HIV-infected individuals, as well as to compare the differences between CRF01_AE and subtype B interpretations.

Materials and Methods

Sample Collection

Stored CRF01_AE plasma samples were selected from individuals who attended clinical sites participating in the TREAT Asia Studies to Evaluate Resistance – Monitoring Study (TASER-M)[Hamers et al., 2011], and can be either from TASER-M or non TASER-M study participants. Samples were chosen either for having been collected at time of treatment failure, or having known primary resistance-associated mutations (RAMs). To maximise the number and diversity of samples available for each site, some samples from antiretroviral therapy (ART)-naïve patients without prior detection of RAMs and with sufficient volume of stored plasma for PT and GT were also chosen. All CRF01_AE samples were shipped to Janssen Diagnostics BVBA for GT, PT and VP. Genotypic sequences in FASTA file format were submitted to the Stanford HIVdb (version 7.0.1) via Sierra – The Stanford HIV Web Service (Version 1.1) for genotypic resistance interpretations. PT and VP were performed using Antivirogram® (assay version 2.5.01) and vircoType™ HIV-1 (version 4.3.01), respectively.

HIV-1 subtype B samples were randomly selected from Janssen Diagnostics BVBA's laboratory databases. To enable comparison with CRF01_AE, where nucleoside and non-nucleoside reverse transcriptase inhibitor (NRTI and NNRTI) RAMs are common[Praparattanapan et al., 2012; Sungkanuparph et al., 2011a; Sungkanuparph et al., 2012], subtype B samples with at least an NRTI and an NNRTI RAMs according to the WHO 2009 mutations list[Bennett et al., 2009], with VP and PT results available, were chosen.

Definition

For direct comparisons between the three methods, resistance interpretations were grouped into binary responses, defined as either “susceptible” (Stanford HIVdb susceptible and potential low-level resistance; vircoType™ HIV-1 susceptible and maximal response; and Antivirogram® susceptible) or “resistant” (Stanford HIVdb low-level, intermediate and high-level resistance; vircoType™ HIV-1 reduced response, minimal response and resistant; and Antivirogram® resistant). A discordant result was defined as having a discrepancy in the binary resistance interpretations between any two methods.

We compared the level of agreement in resistance calls obtained from GT and VP with those obtained from PT in each subtype group. Additionally, the performance of each interpretation system was compared between CRF01_AE and subtype B. This study was not intended in any way to be a comparison of Stanford HIVdb and vircoType™ HIV-1.

As both CRF01_AE and subtype B samples contained transmitted and acquired drug resistance mutations, the prevalence and distribution of RAMs were calculated based on the IAS-USA 2014 mutations list, excluding minor protease inhibitors (PI) RAMs, as these minor variants may occur as common polymorphisms in HIV-1 non-B subtypes[Johnson et al., 2013]. Subtypes were confirmed with vircoType™ HIV-1 clade analysis.

Statistical Analysis

As PT provides a direct measure of ARV resistance, we considered results from Antivirogram® to be the “gold standard” for this study. Comparison of level of agreement between GT and VP predictions with the “gold standard” phenotypes were performed using sensitivity (true positive rate), specificity (true negative rate) and area under the receiver operating characteristics curve (AUC) methods. Tests for equality of AUCs were adjusted for multiple comparisons using Bonferroni correction methods.

The Bland-Altman plots[Altman and Bland, 1983; Bland and Altman, 1986] were extrapolated to allow for visual assessment of the behaviour of PT FC values versus VP FC values. The Bland-Altman plots display the differences in FC values between two methods on the vertical axis, and the average FC values of the two methods on the horizontal axis. The difference was calculated by subtracting the VP FC value from the PT value. The plots allow for inspection of existing biases in the calculated FC values.

All data management and statistical analyses were performed at The Kirby Institute, UNSW Australia (The University of New South Wales), Sydney, Australia, using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA) and STATA software version 13.1 (STATA Corp., College Station, TX, USA). For TASER-M samples, written informed consent was obtained prior to cohort enrolment, and ethics approvals were obtained from UNSW Australia Ethics Committee and institutional review boards at the participating clinical sites and coordinating centre (TREAT Asia/amfAR, Bangkok, Thailand). Other CRF01_AE samples were collected during routine clinical care. Data from HIV-1 B subtypes used in the analysis were proprietary of Janssen Diagnostics BVBA.

Results

HIV-1 CRF01_AE

A total of 113 samples were selected from six TREAT Asia sites in Thailand (60/113, 53%), the Philippines (20/113, 18%), Malaysia (14/113, 12%) and Hong Kong (19/113, 17%). Of 113 samples, 95 were successfully amplified with FASTA files available, and 80 successfully completed PT procedures. We therefore performed our comparisons utilising these 80 samples collected from different individuals infected with HIV-1 CRF01_AE in Asia.

Of these 80 samples, 41 (51%) patients had their samples collected prior to starting ART, while 19 (24%) were on NRTI+PI, 13 (16%) were on NRTI+NNRTI and 26 (33%) were on other ART combinations at the time of specimen collection. A total of 55 patients (69%) had 1 RAM, 47 (59%) had NNRTI RAMs, 32 (40%) had NRTI RAMs, and 17 (21%) had PI-major RAMs. The two most common NNRTI RAMs were V179D (16%) and V106I (15%); the most common NRTI RAMs were D67N (29%) and M184V (26%); and the most common PI-major RAMs were M46I (11%) and I84V (9%).

Out of the 80 samples analysed, 3 samples failed PT for efavirenz, etravirine and darunavir/r, 2 for zidovudine and 1 for nevirapine and lamivudine. Therefore the total number of resistance comparisons for each ARV varied from 77–80 specimens. Table 1 (GT vs PT) shows the comparison of GT with PT resistance interpretations. A total of 17 ARVs were included in the analysis (7 NRTIs, 3 NNRTIs and 7 PIs). The total number of sample discordances for each ARV ranged from 3 to 27 samples. The highest number of discordances was observed for stavudine (27/80, 34%), followed by abacavir and didanosine (21/80 each, 26%). Emtricitabine (3/80, 4%) and lamivudine (3/79, 4%) showed the lowest number of discordances. Overall, GT provided the most accurate resistance results for lopinavir/r (AUC = 0.97, sensitivity = 100.0% and specificity = 94.2%) and darunavir/r (AUC = 0.97, sensitivity = 100.0% and specificity = 93.3%). The lowest AUC was observed for didanosine (AUC = 0.76, sensitivity = 80.0% and specificity = 72.9%).

The comparison of VP interpretations with results from PT is also shown in Table 1 (VP vs PT). The number of discordances ranged from 1/80 (1%) for lopinavir/r to 20/80 (25%) for didanosine. Accuracy in VP resistance predictions was highest for lopinavir/r (AUC = 0.99, sensitivity = 100.0% and specificity = 98.6%) and darunavir/r (AUC = 0.99, sensitivity = 100.0% and specificity = 97.3%), and lowest for didanosine (AUC = 0.81, sensitivity = 90.0% and specificity = 72.9%). With the exception of didanosine, the sensitivities for all other NRTIs were 100%, suggesting that VP was able to correctly classify patients as truly resistant for these NRTI drugs.

As didanosine had the highest number of discordances and the lowest AUC, we chose to construct the Bland-Altman plot for this ARV based on the FC values obtained from both VP and PT, in order to investigate the biases associated with VP resistance predictions. Overall, Figure 1(a) shows scattered points above and below 0 with a possible increasing trend at low FC values, and an outlier at the average value of 6.3. This indicates that at higher susceptibility levels (average FC of less than 1), VP tends to result in larger FC

predictions, compared to PT, causing the plot to consist mainly of negative values. However, as resistance increases, up to the average FC of approximately 1.5 on the horizontal axis, the VP predicted FC became smaller than PT, but this trend was not seen at larger FC values. For the sample that had the highest difference between PT FC and VP FC values, i.e the outlier, the PT FC value was 2.8 (resistant) and the VP FC value was 9.8 (minimal response). Although both methods provided “resistant” predictions to didanosine for this sample, the difference between the two FC values were substantially larger in comparison to other samples. Protease (PR) RAMs detected from this outlier sample were M46I, I47V and I84V, and reverse transcriptase (RT) RAMs were A62V, D67N, K70R, V75I, F116Y, Q151M and K219Q. This sample was the only CRF01_AE sample with the Q151M mutation which is known to be associated with multiple NRTI drug resistance [Johnson et al., 2013].

HIV-1 Subtype B

PT and VP results for 100 HIV-1 subtype B samples from different individuals were analysed. No ART information was available for these patients. All 100 patients had 1 RAM with the following proportions: 100% had NNRTI RAMs, 95% had NRTI RAMs and 42% had PI-major RAMs. The most common NNRTI RAM was K103N (65%) followed by Y181C (25%). M184V (71%) and M41L (30%) were the two most common NRTI RAMs, and L90M (24%) and V82A (17%) the two most common PI-major RAMs.

GT resistance interpretations compared to PT are shown in Table 2 (GT vs PT). Of 100 subtype B samples, the most number of discrepancies was observed for didanosine (61/100, 61%) with AUC of 0.56, sensitivity = 82.4% and specificity = 30.1. Abacavir, however, showed the lowest AUC of 0.55, sensitivity = 100.0% and specificity = 9.1%, with 60/100 samples (60%) having discordant interpretations. PI drug class showed 100% sensitivity for all ARVs reported in that class. The AUC for efavirenz and nevirapine were not calculated due to the absence of GT susceptible predictions.

Table 2 (VP vs PT) shows the comparison of drug resistance interpretations between VP and PT. The total number of discordances in resistance interpretations ranged from 2/100 (2%) for saquinavir/r to 75/100 (75%) for didanosine. As expected, the accuracy of VP predictions was highest for saquinavir/r (AUC = 0.97, sensitivity = 96.2% and specificity = 98.7%) and lowest for didanosine (AUC = 0.55, sensitivity = 100.0% and specificity = 9.6%).

The Bland-Altman plot for didanosine (Figure 1b), shows a similar pattern to that of CRF01_AE. There appears to be mostly negative differences around the average FC of 1, suggesting that VP FC values were mainly larger than PT FC. The plot then becomes scattered above and below the difference of 0 as the average FC increases, indicating that no trend is observed in the differences between the two methods for large FC values. The outlier occurred at PT FC of 5.9 (resistant) and VP FC of 13.3 (minimal response). The PR RAMs for this sample were V32I, I47V, I54L, V82A and L90M. RT RAMs were A62V, D67N, V75I, F77L, K101H, Y115F, F116Y, Q151M, Y181C, G190A and K219E. This was the only sample harbouring Q151M mutation out of all subtype B samples.

Comparison of the level of agreement between CRF01_AE and subtype B

We examined the level of agreement between CRF01_AE and subtype B samples by testing for equality of the AUCs as shown in Table 3. In terms of VP predictions, VP provided better predicted resistance in CRF01_AE than in subtype B for efavirenz (0.87 vs 0.64, $p=0.024$), abacavir (0.87 vs 0.55, $p<0.001$), didanosine (0.81 vs 0.55, $p<0.001$), emtricitabine (0.98 vs 0.82, $p=0.007$), and tipranavir/r (0.97 vs 0.93, $p=0.035$). For GT comparisons, again CRF01_AE performed better than subtype B for etravirine (0.88 vs 0.77, $p=0.041$), abacavir (0.85 vs 0.55, $p<0.001$), didanosine (0.76 vs 0.56, $p=0.025$), stavudine (0.83 vs 0.71, $p=0.002$), tenofovir (0.86 vs 0.77, $p=0.013$), zidovudine (0.93 vs 0.84, $p=0.007$), lopinavir/r (0.97 vs 0.91, $p=0.018$) and tipranavir (0.94 vs 0.88, $p=0.035$). After Bonferroni adjustment for multiple comparisons, VP performed significantly better in CRF01_AE only for abacavir and didanosine, and similarly for GT for abacavir and stavudine, at a Bonferroni adjusted significance level of 0.003.

Discussion

Our study found that GT resistance predictions and VP resistance predictions were highly concordant compared to results obtained from the gold standard PT for our CRF01_AE sample group. The number of discordances ranged from 3 to 27 samples for GT and 1 to 20 samples for VP. The AUCs for both interpretation systems were also high, ranging from 0.76 to 0.97 for GT and 0.81 to 0.99 for VP. The Bland-Altman plot for didanosine, the ARV with the highest VP discrepancy, showed that at very low FC values, the predicted VP FC values were larger than the PT FC values. However, this pattern was not seen as resistance increased. Subtype B samples showed similarly high level of agreement with the exception of abacavir and didanosine which had the lowest AUCs for both VP and GT comparisons. The pattern of the Bland-Altman plot for didanosine for subtype B samples was similar to that of CRF01_AE samples. An outlier existed in each of the subtype groups with the associated sample being the only one for each subtype to harbour the multiple NRTI drug resistance mutation Q151M. Comparison of the AUCs for VP and GT predictions showed that CRF01_AE samples consistently performed better than subtype B for both interpretation systems.

The overall high level of agreement between the different drug resistance interpretation systems found in this study is consistent with previous findings[Jiamsakul et al., 2012; Liu et al., 2008; Muñoz et al., 2005; Van Houtte et al., 2009]. However, the results of our study also suggest that the level of accuracy may vary with different subtypes. As drug resistance algorithms commonly use subtype B as the consensus sequence, it was expected that these algorithms would provide more accurate predictions for subtype B samples. Studies have shown that when comparing resistance predictions derived from commonly used HIV drug resistance interpretation algorithms, non-B subtypes normally resulted in higher number of discordances compared to subtype B. A possible explanation is that resistance algorithms are subtype dependent on many drugs[Snoeck et al., 2006; Sturmer et al., 2011]. Interestingly, our study found that predictions for CRF01_AE were significantly more accurate than subtype B for abacavir (GT and VP), didanosine (VP), and stavudine (GT), whilst no significant differences were found for other ARVs. This is of particular importance to

resource-limited settings within Asia such as those of TASER-M clinical sites, as CRF01_AE has been reported as the predominant subtype in the TASER-M cohort and within the region[Angelis et al., 2015; Hamers et al., 2012; Sungkanuparph et al., 2011b]. The findings reinforce the suitability of the application of both resistance interpretation systems on sequences derived from HIV-infected patients in Asia. Consequently, clinicians from resource-limited settings can be presented with various options for analysing RAMs without compromising on the level of accuracy. It should also be noted that although interpretations for CRF01_AE were unexpectedly more accurate than subtype B for three of the ARVs, our study should not be interpreted as evidence to indicate superiority of CRF01_AE over subtype B in these resistance algorithms. The variation in accuracy between subtypes for different drugs is similar to that found in a previous Thai study where subtype B and CRF01_AE were compared using seven-rule based algorithms[Poonpiriya et al., 2008]. The study also found that didanosine had the poorest agreement for both subtype groups.

From the Bland-Altman plot, the sample that showed the largest difference between the predicted virtual phenotype FC value and the laboratory-based phenotype FC value for didanosine, i.e the outlier sample for each subtype, was the only sample for that subtype group to contain the Q151M mutation. That is, only one CRF01_AE sample and one subtype B sample analysed in our study, both of which showed the biggest differences in didanosine FC values, were found to have the Q151M RAM. This RAM was absent in all other samples. The Q151M complex, consisting of Q151M, A62V, V75I, F77L and F116Y, is associated with resistance to all approved NRTIs except for tenofovir[Johnson et al., 2013]. Q151M is the most important mutation in this complex as other mutations alone may not cause multiple drug resistance.

A study of CRF01_AE HIV-infected Cambodian patients found the Q151M mutation to be associated with stavudine-use[Nouhin et al., 2013]. On the contrary, the presence of Q151M was not associated with any predictors in subtype B HIV-infected patients from the Swiss HIV Cohort Study[Scherrer et al., 2011]. Our CRF01_AE patient harbouring the Q151M mutation was treated with didanosine, zidovudine and boosted lopinavir at the time of sample collection. Therefore we could not confirm the possible association between Q151M and stavudine exposure using our sample. Although Q151M was found in two patients with the highest didanosine FC differences, our study could not directly support the possible link between Q151M and the highly discordant didanosine FC values. The level of didanosine susceptibility for both patients was reported as “resistant” from PT and “minimal response” from VP. Both of which could be further grouped as “resistant”. The subtype B sample contained all mutations in the Q151M complex while the CRF01_AE sample lacked the F77L mutation. Other RT RAMs found in the CRF01_AE sample were thymidine analogue-associated mutations (TAMs) which included D67N, K70R and K219Q. Two TAMs were also present in the subtype B sample (D67N and K219E). The presence of Q151M and TAMs in these two samples indicate multiple NRTI drug resistance, which supports our “resistant” interpretation results for didanosine. Because resistance algorithms can be affected by specific mutation combinations, it is possible that the combination of Q151M and TAMs found in these two samples may have partly influence the predicted resistance calls derived from the algorithm. It is therefore important to take into consideration the FC

values together with the resistance interpretations, and mutations when evaluating HIV drug resistance.

The main limitations of this study included the unavailability of patient demographics, clinical characteristics and treatment outcome measurements for the majority of the samples used in the analysis. We therefore were unable to measure the relationship between these characteristics and the discrepancies observed in the resistance algorithms. We were also unable to investigate the effects of these discrepancies on subsequent treatment responses due to the lack of patient data. Because of the absence of information on ART for subtype B patients, we could not perform direct comparison with CRF01_AE patients of the same ART exposure. The small sample sizes for both CRF01_AE and subtype B also means that our findings may not be applicable to other non-B subtypes or the general HIV-infected population.

In conclusion, GT and VP provided high level agreement in their resistance interpretations in comparison to PT for both CRF01_AE and B subtypes in our study. GT and VP predictions were more accurate for CRF01_AE for three NRTIs. Although discrepancies were noted, GT and VP resistance algorithms developed mainly from subtype B viruses were highly robust in their predictions of ARV resistance for HIV-1 CRF01_AE.

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Figure 1.

Bland-Altman plot for didanosine for the comparison of laboratory-based phenotyping Fold Change values (PT FC) with virtual phenotyping Fold Change values (VP FC) for (a) HIV-1 CRF01_AE and (b) HIV-1 subtype B infected persons. A point above 0 on the vertical axis suggests that the FC obtained from PT is higher than the predicted FC from VP, whereas a point below 0 indicates the predicted VP FC is higher. 95% CI – 95% confidence interval.

Table 1
Comparison of genotyping and virtual phenotyping with laboratory-based phenotyping for HIV-1 CRF01_AE infected persons.

GT vs PT										VP vs PT									
Concordant					Discordant					Concordant					Discordant				
Total					Total					Total					Total				
Drug Class	Drug	Total	GT-S/PT-S	GT-R/PT-R	GT-R/PT-S	GT-S/PT-R	Dis	Sen	Spec	AUC	VP-S/PT-S	VP-R/PT-R	VP-R/PT-S	VP-S/PT-R	Dis	Sen	Spec	AUC	
NNRTI	Efavirenz	77	41	26	0	10	10	72.2%	100.0%	0.86	36	31	5	5	10	86.1%	87.8%	0.87	
	Etravirine	77	54	12	10	1	11	92.3%	84.4%	0.88	57	12	7	1	8	92.3%	89.1%	0.91	
	Nevirapine	79	39	27	6	7	13	79.4%	86.7%	0.83	41	27	4	7	11	79.4%	91.1%	0.85	
NRTI	Abacavir	80	49	9	22	0	22	100.0%	69.0%	0.85	52	9	19	0	19	100.0%	73.2%	0.87	
	Didanosine	80	51	8	19	2	21	80.0%	72.9%	0.76	51	9	19	1	20	90.0%	72.9%	0.81	
	Emtricitabine	80	52	25	2	1	3	96.2%	96.3%	0.96	52	26	2	0	2	100.0%	96.3%	0.98	
	Lamivudine	79	52	22	4	1	5	95.7%	92.9%	0.94	51	23	5	0	5	100.0%	91.1%	0.96	
	Stavudine	80	51	2	27	0	27	100.0%	65.4%	0.83	64	2	14	0	14	100.0%	82.1%	0.91	
	Tenofovir	80	53	7	20	0	20	100.0%	72.6%	0.86	56	7	17	0	17	100.0%	76.7%	0.88	
	Zidovudine	78	52	18	8	0	8	100.0%	86.7%	0.93	52	18	8	0	8	100.0%	86.7%	0.93	
PI	Atazanavir/r	80	62	13	3	2	5	86.7%	95.4%	0.91	64	14	1	1	2	93.3%	98.5%	0.96	
	Darunavir/r	77	69	2	6	0	6	100.0%	92.0%	0.96	73	2	2	0	2	100.0%	97.3%	0.99	
	Indinavir/r	80	63	12	4	1	5	92.3%	94.0%	0.93	64	13	3	0	3	100.0%	95.5%	0.98	
	Lopinavir/r	80	65	11	4	0	4	100.0%	94.2%	0.97	68	11	1	0	1	100.0%	98.6%	0.99	
	Nelfinavir	80	62	15	2	1	3	93.8%	96.9%	0.95	62	15	2	1	3	93.8%	96.9%	0.95	
	Saquinavir/r	80	63	10	6	1	7	90.9%	91.3%	0.94	68	10	1	1	2	90.9%	98.6%	0.95	
	Tipranavir/r	80	68	3	9	0	9	100.0%	88.3%	0.94	73	3	4	0	4	100.0%	94.8%	0.97	

Abbreviations: GT - genotyping; VP - virtual phenotyping; PT - laboratory-based phenotyping; GT-S - Stanford HIVdb susceptible and potential low-level resistance; VP-S - vircoTypeTM HIV-1 susceptible and maximal response; PT-S - Antivirogram[®] susceptible; GT-R - Stanford HIVdb low-level, intermediate and high-level resistance; VP-R - vircoTypeTM HIV-1 reduced response, minimal response and resistant; PT-R - Antivirogram[®] resistant; Dis - discordances; Sen - sensitivity; Spec - specificity; AUC - area under the curve; NRTI - nucleoside reverse transcriptase inhibitor; NNRTI - non-NRTI; and PI - protease inhibitor.

Table 2
Comparison of genotyping and virtual phenotyping with laboratory-based phenotyping for HIV-1 subtype B infected persons.

GT vs PT											VP vs PT																							
Concordant					Discordant					Total					Concordant					Discordant					Total					AUC				
Drug Class	Drug	Total	GT-S/PT-S	GT-R/PT-R	GT-R/PT-S	GT-S/PT-R	Dis	Sen	Spec	AUC	Concordant					Discordant					Total					AUC								
NNRTI	Efavirenz	100	0	93	7	0	0	7	100.0%	N/A	2	93	5	0	5	100.0%	28.6%	0.64																
	Etravirine	100	33	40	26	1	27	97.6%	55.9%	0.77	48	38	11	3	14	92.7%	81.4%	0.87																
	Nevirapine	100	0	97	3	0	3	100.0%	N/A	N/A	0	97	3	0	3	100.0%	N/A	N/A																
NRTI	Abacavir	100	6	34	60	0	60	100.0%	9.1%	0.55	7	34	59	0	59	100.0%	10.6%	0.55																
	Didanosine	100	25	14	58	3	61	82.4%	30.1%	0.56	8	17	75	0	75	100.0%	9.6%	0.55																
	Emtricitabine	100	13	81	5	1	6	98.7%	72.2%	0.86	12	80	6	2	8	97.6%	66.7%	0.82																
	Lamivudine	100	13	84	2	1	3	98.8%	86.7%	0.93	11	85	4	0	4	100.0%	73.3%	0.87																
	Stavudine	100	36	13	51	0	51	100.0%	41.4%	0.71	74	13	13	0	13	100.0%	85.1%	0.93																
	Tenofovir	100	48	11	41	0	41	100.0%	53.9%	0.77	56	11	33	0	33	100.0%	62.9%	0.81																
	Zidovudine	100	45	33	22	0	22	100.0%	67.2%	0.84	60	33	7	0	7	100.0%	89.6%	0.95																
PI	Atazanavir/r	100	59	32	9	0	9	100.0%	86.8%	0.93	65	31	3	1	4	96.9%	95.6%	0.96																
	Darunavir/r	100	83	8	9	0	9	100.0%	90.2%	0.95	89	7	3	1	4	87.5%	96.7%	0.92																
	Indinavir/r	100	60	32	8	0	8	100.0%	88.2%	0.94	66	31	2	1	3	96.9%	97.1%	0.97																
	Lopinavir/r	100	63	23	14	0	14	100.0%	81.8%	0.91	71	23	6	0	6	100.0%	92.2%	0.96																
	Nelfinavir	100	57	33	10	0	10	100.0%	85.1%	0.93	62	33	5	0	5	100.0%	92.5%	0.96																
	Saquinavir/r	100	62	26	12	0	12	100.0%	83.8%	0.92	73	25	1	1	2	96.2%	98.7%	0.97																
	Tipranavir/r	100	66	13	21	0	21	100.0%	75.9%	0.88	74	13	13	0	13	100.0%	85.1%	0.93																

Abbreviations: GT - genotyping; VP - virtual phenotyping; PT - laboratory-based phenotyping; GT-S - Stanford HIVdb susceptible and potential low-level resistance; VP-S - vircoTypeTM HIV-1 susceptible and maximal response; PT-S - Antivirogram[®] susceptible; GT-R - Stanford HIVdb low-level, intermediate and high-level resistance; VP-R - vircoTypeTM HIV-1 reduced response, minimal response and resistant; PT-R - Antivirogram[®] resistant; Dis - discordances; Sen - sensitivity; Spec - specificity; AUC - area under the curve; NRTI - nucleoside reverse transcriptase inhibitor; NNRTI - non-NRTI; and PI - protease inhibitor.

Table 3

Comparison of AUC values for virtual phenotype and genotype interpretations between CRF01_AE and subtype B.

Drug Class	Drug	AUC VP compared to PT			AUC GT compared to PT		
		CRF01_AE	Subtype B	p-value	CRF01_AE	Subtype B	p-value
NNRTI	Efavirenz	0.87	0.64	0.024	0.86	N/A	N/A
	Etravirine	0.91	0.87	0.499	0.88	0.77	0.041
	Nevirapine	0.85	N/A	N/A	0.83	N/A	N/A
NRTI	Abacavir	0.87	0.55	<0.001	0.85	0.55	<0.001
	Didanosine	0.81	0.55	<0.001	0.76	0.56	0.025
	Emtricitabine	0.98	0.82	0.007	0.96	0.86	0.071
	Lamivudine	0.96	0.87	0.154	0.94	0.93	0.778
	Stavudine	0.91	0.93	0.606	0.83	0.71	0.002
	Tenofovir	0.88	0.81	0.054	0.86	0.77	0.013
	Zidovudine	0.93	0.95	0.620	0.93	0.84	0.007
PI	Atazanavir/r	0.96	0.96	0.933	0.91	0.93	0.648
	Darunavir/r	0.99	0.92	0.305	0.96	0.95	0.688
	Indinavir/r	0.98	0.97	0.726	0.93	0.94	0.835
	Lopinavir/r	0.99	0.96	0.062	0.97	0.91	0.018
	Nelfinavir	0.95	0.96	0.795	0.95	0.93	0.485
	Saquinavir/r	0.95	0.97	0.596	0.94	0.92	0.883
	Tipranavir/r	0.97	0.93	0.035	0.94	0.88	0.035

Abbreviations: VP – virtual phenotyping; PT – laboratory-based phenotyping; GT – genotyping; NRTI – nucleoside reverse transcriptase inhibitor; NNRTI – non-NRTI; PI – protease inhibitor; and AUC – area under the curve (AUC of 1.0 implies perfect accuracy and AUC of 0.5 indicates the system is no better than a random prediction).