Comparison of the Precision and Sensitivity of the Antivirogram and PhenoSense HIV Drug Susceptibility Assays

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Objective: Although 2 widely used susceptibility assays have been developed, their precision and sensitivity have not been assessed.

Design and Methods: To assess the precision of the Antivirogram and PhenoSense assays, we examined susceptibility results of HIV-1 isolates lacking drug resistance mutations and containing matching patterns of drug resistance mutations. To assess sensitivity, we determined for each assay the proportion of isolates with common patterns of matching drug resistance mutations having reductions in susceptibility greater than those in isolates without drug resistance mutations.

Results: We analyzed protease inhibitor (PI) susceptibility results obtained by the Antivirogram assay for 293 isolates and by the PhenoSense assay for 300 isolates. We analyzed reverse transcriptase (RT) inhibitor susceptibility results obtained by the Antivirogram assay for 202 isolates and by the PhenoSense assay for 126 isolates. For wild-type and mutant isolates, the median absolute deviance of the fold resistance of nucleoside RT inhibitor susceptibility results was significantly lower for the PhenoSense assay than for the Antivirogram assay. The PhenoSense assay was also significantly more likely than the Antivirogram assay to detect resistance to abacavir, didanosine, and stavudine in isolates with the common drug resistance mutations M41L, M184V, and T215Y (±L210W). We found no significant differences between the 2 assays for detecting PI and nonnucleoside RT inhibitor resistance.

Conclusion: The PhenoSense assay is more precise than the Antivirogram assay and superior at detecting resistance to abacavir, didanosine, and stavudine.

Key Words: antiretroviral therapy, HIV reverse transcriptase, HIV protease, drug resistance, sensitivity, specificity

(*J Acquir Immune Defic Syndr* 2005;38:439–444)

Received for publication May 15, 2004; accepted September 28, 2004.

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J. Zhang and R. W. Shafer were supported in part by a grant from the National Institutes of Allergy and Infectious Diseases (AI46148-01). S.-Y. Rhee, J. Taylor, and R. W. Shafer were supported in part by a grant from the National Institute of General Medical Sciences (5P01GM066524-02).

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Because accurate and reproducible phenotypic susceptibility results are essential to the advancement of HIV-1 drug resistance knowledge and the clinical management of HIV-1–infected patients, we compared the precision and sensitivity of the 2 most widely used drug susceptibility assays—Antivirogram (Virco, Mechelin, Belgium) and PhenoSense (ViroLogic, South San Francisco, CA)—to determine whether they are equally effective at detecting drug resistance in viruses with common combinations of drug resistance mutations. Both assays are intricate, high-throughput, commercially available tests performed in specialized facilities within each company.

METHODS

HIV-1 Isolates

We selected all isolates in the Stanford HIV reverse transcriptase (RT) and Protease Sequence Database for which drug susceptibility results were determined by the Antivirogram or PhenoSense assay.[1] A complete listing of the isolates and the studies in which they were published can be found at http://hivdb.stanford.edu/pages/publications.html. Isolates were included if they lacked drug resistance mutations or if they contained a pattern of drug resistance mutations present in 2 or more isolates tested by each assay. Viruses obtained from patient plasma were classified as clinical isolates. Viruses with mutations resulting from in vitro virus passage or site-directed mutagenesis were classified as laboratory isolates. Although susceptibility data of laboratory and clinical isolates were pooled, all statistically significant findings were also present in analyses that excluded laboratory isolates. Isolates with mixed quasispecies at major drug resistance positions were excluded from analysis.

Drug Resistance Mutation Patterns

Protease inhibitor (PI) resistance mutation patterns were defined by mutations at 14 nonpolymorphic protease positions associated with PI resistance: 24, 30, 32, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, and 90. RT inhibitor resistance mutation patterns were defined by mutations at 33 RT positions, including 18 positions associated with nucleoside reverse transcriptase inhibitor (NRTI) resistance (41, 44, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 118, 151, 184, 210, 215, and 219) and 15 positions associated with nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance (98, 100, 101, 103, 106, 108, 179, 181, 188, 190, 225, 227, 230, 236, and 238). Drug

resistance mutations at polymorphic protease positions (10, 20, 33, 36, 63, 71, 77, and 93) were not used to define PI mutation patterns. Two common polymorphisms at NNRTI resistance positions A98S and V179I were not used to define NNRTI mutation patterns.

Sequences containing a mixture of amino acids at NRTI resistance positions 41, 65, 67, 69, 70, 74, 75, 151, 184, 210, 215, and 219; at NNRTI resistance positions 100, 101, 103, 106, 181, 188, 190, 225, 230, and 238; and at PI resistance positions 30, 48, 50, 82, 84, and 90 were excluded from analysis. Sequences containing a mixture at more than a third of all drug resistance positions for a given drug class were also excluded from analysis.

Analysis

Susceptibility results were expressed as fold resistance compared with laboratory wild-type controls. Sufficient susceptibility data for analysis were available for 15 of 18 approved RT inhibitors and PIs. Wild-type isolates were defined as lacking mutations affecting the class of drug used in the susceptibility assay. Mutant isolates were defined as having 1 or more mutations affecting the class of the drug used in the susceptibility assay.

The precision of each assay was assessed by comparing the median absolute deviance from the median (ie, the median absolute deviance) of susceptibility results on wild-type isolates and isolates with matching patterns of drug resistance mutations. The residuals of each assay (from the median) were compared using a weighted rank sum test to identify significant differences between the assays in the median absolute deviance of susceptibility results for isolates with identical patterns of drug resistance mutations (Fligner test).[2] Probability values less than 0.0033 (Bonferroni adjustment for 15 drug comparisons) were considered statistically significant.

In addition to this nonparametric method, we assessed precision using the F-test to identify significant differences between the assays in the standard deviation of susceptibility results for isolates with identical drug resistance mutation patterns. Comparisons were performed on log-transformed levels of fold resistance to stabilize variance and render the data approximately normal to enable pooling of results from isolates with different patterns of drug resistance mutations. Although parametric and nonparametric comparisons were done, only the nonparametric results are presented. All statistically significant differences between the assays found using nonparametric methods were also found using parametric methods.

The sensitivity of each assay was determined by calculating the proportion of isolates with common patterns of drug resistance mutations that had levels of fold resistance exceeding the "biologic cutoff" for that drug—a measurement based on the fold resistance of wild-type isolates.[3,4] The biologic cutoff was calculated by taking the exponent of the mean plus 2 standard deviations greater than the mean of the log-transformed data. For certain drugs, additional biologic cutoffs, including those previously published for each of the assays,[3,4] were explored by means of a receiver operator characteristic (ROC) analysis using a bootstrap estimate of standard error.[5]

RESULTS

Source of Isolates

A total of 594 RT and 542 protease isolates tested by the Antivirogram assay were available. Of these, 94 RT and 28 protease isolates were laboratory isolates. One hundred thirty-four RT and 96 protease isolates were excluded from analysis because of the presence of mixtures at 1 or more drug resistance positions (see Methods section). Twelve protease and 7 RT isolates tested by the Antivirogram assay were non-subtype B isolates. A total of 522 RT and 678 protease isolates tested by the PhenoSense assay were available. Of these, 63 RT and 54 protease isolates were laboratory isolates. One hundred thirty-two RT and 72 protease isolates were excluded because of the presence of mixtures at 1 or more drug resistance positions. Two protease and no RT isolates tested by the PhenoSense assay were non-subtype B isolates.

Isolates Without Drug Resistance Mutations

The median absolute deviance of the fold resistance of the log-transformed NRTI susceptibility results was significantly lower for the PhenoSense assay than for the Antivirogram assay for every NRTI except zidovudine (Table 1). With the PhenoSense assay, the median absolute deviance of the log-transformed values for lamivudine, abacavir, stavudine, zalcitabine, and didanosine was 0.04 to 0.06 or 1.1-fold ($10^{0.04}$–$10^{0.06}$). With the Antivirogram assay, the median absolute deviance for the same drugs was 0.12 to 0.23 (1.3–1.7-fold).

Although the median absolute deviance of the fold resistance of the log-transformed PI susceptibility results was significantly lower for the PhenoSense assay than for the Antivirogram assay for indinavir, nelfinavir, ritonavir, and saquinavir, the difference was not as great as for the NRTIs. The median absolute deviance of the log-transformed values for these 4 PIs was 0.10 to 0.14 (1.3–1.4-fold) for the PhenoSense assay and 0.18 to 0.26 (1.5–1.8-fold) for the Antivirogram assay. The median absolute deviance of the fold resistance of the log-transformed NNRTI susceptibility results for the 2 assays was similar.

Isolates With Matching Patterns of Drug Resistance Mutations

A summary of drug susceptibility results on isolates with matching patterns of drug resistance mutations is shown in Table 2. Isolates with matching patterns of drug resistance mutations often differed at non-drug resistance positions. The numbers of mutations at these positions in isolates tested by the Antivirogram and PhenoSense assays were similar, however. The median number of non-drug resistance RT mutations between positions 1 and 300 was 6 (interquartile range: 4–8) for the Antivirogram assay and 7 (interquartile range: 4–10) for the PhenoSense assay. The median number of non-drug resistance protease mutations between positions 1 and 99 was 7 (interquartile range: 5–9) for the Antivirogram assay and 6 (interquartile range: 3–7) for the PhenoSense assay.

Results for 8 to 10 matching patterns (depending on the drug) of NRTI resistance mutations were available on 50 to 61 isolates tested by the PhenoSense assay and on 70 to 93

TABLE 1. Nonparametric Measures of Reproducibility of Susceptibility Tests Performed on HIV-1 Isolates Lacking Drug Resistance Mutations: Comparison of the Antivirogram and PhenoSense Assays

	Number		Median		Median Absolute Deviance		
Drug	**AV**	**PS**	**AV**	**PS**	**AV**	**PS**	***P***
NRTIs							
3TC	83	45	0.8	1.0	**0.20**	**0.05**	**6E-08**
ABC	80	45	0.75	0.8	**0.24**	**0.06**	**3E-08**
D4T	88	45	0.8	1.0	**0.20**	**0.05**	**7E-10**
DDC	78	38	0.9	1.0	**0.12**	**0.04**	**5E-08**
DDI	88	45	0.8	1.0	**0.19**	**0.05**	**1E-10**
ZDV	88	45	1.2	0.8	0.11	0.10	2E-01
PIs							
APV	118	104	0.6	0.8	0.18	0.12	2E-02
IDV	146	134	0.75	0.9	**0.18**	**0.10**	**1E-06**
LPV	72	54	0.75	0.8	0.13	0.12	3E-01
NFV	144	137	0.9	1.3	**0.26**	**0.14**	**5E-05**
RTV	146	134	0.7	1.0	**0.24**	**0.13**	**4E-06**
SQV	145	131	0.7	0.8	**0.20**	**0.12**	**1E-07**
NNRTIs							
DLV	43	47	2.5	1.3	0.24	0.25	8E-01
EFV	45	47	1.3	0.9	0.21	0.12	4E-02
NVP	83	47	1.4	0.9	0.24	0.18	3E-01

The Fligner test was used to identify significant differences in the median absolute deviance of results determined by AV and PS assays. The results of this test and the median and median absolute deviance were determined using log-transformed values of fold resistance. The antilog of the median is shown in the table. Significant results ($P < 0.0033$), based on a Bonferroni adjustment for 15 comparisons, are in bold. One hundred and fifty and 137 wild-type protease isolates were tested by ≥1 PI by AV and PS assays, respectively. Ninety-one and 47 wild-type RT isolates were tested by ≥1 RT inhibitor by AV and PS assays, respectively.

ABC, abacavir; APV, amprenavir; AV, Antivirogram assay; D4T, stavudine; DDC, zalcitabine; DDI, didanosine; DLV, delavirdine; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; PS, PhenoSense assay; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; ZDV, zidovudine.

isolates tested by the Antivirogram assay. The median absolute deviance for lamivudine was not calculated, because results on a large proportion of the isolates exceeded the upper limits of both assays. The most common RT mutation patterns in isolates tested by both assays included M184V (75 isolates), M41L + M184V + T215Y (24 isolates), M41L + M184V + L210W + T215Y (15 isolates), K103N + M184V (11 isolates), and K65R (7 isolates). The median absolute deviance of the log-transformed levels of fold resistance to abacavir, didanosine, stavudine, and zalcitabine was significantly lower for the PhenoSense assay (0.05–0.08 or 1.1–1.2-fold) than for the Antivirogram assay (0.15–0.23 or 1.4–1.7-fold).

Results for 15 to 28 matching patterns of PI resistance mutations were available on 65 to 136 isolates tested by the Antivirogram assay and on 46 to 161 isolates tested by the PhenoSense assay. The most common patterns tested by both assays included D30N + N88D (45 isolates), L90M (25 isolates), D30N (19 isolates), I54V + V82A + L90M (16 isolates), and M46L + I54V + V82A + L90M (12 isolates). The median absolute deviance of the log-transformed levels of fold resistance to ritonavir was significantly lower for the Pheno Sense assay than for the Antivirogram assay.

Results for 10 to 12 matching patterns of NNRTI resistance mutations were available on 51 to 90 isolates tested by the Antivirogram assay and for 59 to 67 isolates tested by the PhenoSense assay. The most common NNRTI mutation patterns in isolates tested by each assay included K103N + M184V (11 isolates), K103N (9 isolates), and Y181C (6 isolates). There were no significant differences in the median absolute deviance of the log-transformed levels of fold resistance to the NNRTIs between the Antivirogram and PhenoSense assays.

Assay Sensitivity

To examine the sensitivity of the Antivirogram and PhenoSense assays at detecting drug resistance, we compared the proportion of isolates with common drug resistance mutation patterns that had levels of resistance higher than each assay's biologic cutoff for each drug. The biologic cutoffs for the NRTIs were lower for the PhenoSense assay (1.32–1.87-fold) than for the Antivirogram assay (2.75–3.80-fold). For each assay, the proportion of isolates with the most common NRTI resistance mutation patterns with susceptibility results exceeding the biologic cutoffs for abacavir, didanosine, lamivudine, stavudine, and zidovudine is shown in Table 3.

Both assays assigned a level of resistance to lamivudine above the biologic cutoffs whenever M184V was present. The PhenoSense assay was significantly more likely than the Antivirogram assay to detect resistance above the biologic cutoffs for abacavir and didanosine in these isolates, however. Both assays assigned zidovudine resistance to isolates with M41L + T215Y + M184V or M41L + L210W + T215Y + M184V. In these isolates, the PhenoSense assay was significantly more likely than the Antivirogram assay to detect levels of resistance to abacavir, didanosine, and stavudine above these drugs' biologic cutoffs.

Figure 1 shows ROC curves demonstrating the tradeoff between sensitivity and specificity for the Antivirogram and PhenoSense assays in detecting decreased susceptibility to abacavir, didanosine, and stavudine in isolates containing M184V alone and M41L + M184V + T215Y ± L210W. These curves show that regardless of whether the biologic cutoff is the geometric mean plus 2 standard deviations, the 98th percentile fold change, or the previously published biologic cutoff for these drugs,[3,4] the PhenoSense assay is significantly more sensitive than the Antivirogram assay in detecting decreased NRTI susceptibility. Indeed, tests of differences between the areas under the empiric ROC curves showed that the PhenoSense assay was significantly more sensitive than the Antivirogram assay for abacavir ($P = 0.02$), didanosine ($P < 0.001$), and stavudine ($P < 0.001$) for M41L + M184V + T215Y ± L210W and for abacavir ($P < 0.001$) and didanosine ($P < 0.001$) for M184V alone.[5]

The biologic cutoffs for the PIs were similar for the Antivirogram (1.64–3.83-fold) and PhenoSense (1.62–3.45-fold) assays, and no differences were found for detecting PI resistance. Both assays found that all isolates with D30N ± N88D had reduced nelfinavir susceptibility and that isolates with D30N alone (but not necessarily D30N + N88D) were susceptible to each of the remaining PIs. Isolates with L90M alone had a median level of resistance that was close to the

TABLE 2. Nonparametric Measures of Reproducibility of Susceptibility Tests Performed on HIV-1 Isolates with Matching Patterns of Drug Resistance Mutations: Comparison of the Antivirogram and PhenoSense Assays

		No. Isolates		Median*		Median Absolute Deviance		
Drug	**No. Patterns**	**AV**	**PS**	**AV**	**PS**	**AV**	**PS**	***P*†**
NRTIs								
3TC	10	93	58	ND	ND	ND	ND	ND
ABC	9	70	61	0.9–6.0	1.0–8.0	**0.19**	**0.08**	**9E-07**
D4T	10	90	60	0.6–2.8	0.8–3.3	**0.15**	**0.05**	**3E-10**
DDC	8	84	50	0.8–4.2	1.1–3.1	**0.20**	**0.04**	**2E-10**
DDI	10	89	58	0.4–4.0	0.9–2.1	**0.23**	**0.04**	**2E-11**
ZDV	10	93	58	0.8–68	0.4–354	0.16	0.10	2E-02
PIs								
APV	24	91	112	0.4–6.0	0.1–21	0.19	0.12	5E-03
IDV	28	134	160	0.4–45	0.7–53	0.17	0.11	1E-02
LPV	15	65	46	0.5–24	0.3–33	0.15	0.09	1E-01
NFV	27	125	159	0.5–48	1.5–74	0.18	0.16	9E-01
RTV	25	127	148	0.3–134	0.6–83	**0.24**	**0.10**	**4E-07**
SQV	28	136	161	0.3–130	0.5–241	0.16	0.13	3E-02
NNRTIs								
DLV	12	72	64	0.6–157	0.2–89	0.18	0.15	1E-01
EFV	10	51	59	0.7–141	0.3–76	0.19	0.10	3E-02
NVP	12	90	67	0.9–414	0.32–249	0.16	0.12	9E-02

*Median was calculated independently for each pattern of mutations. Results for patterns with lowest and highest medians are shown.

†The Fligner test was used to identify significant differences in the median absolute deviance of the AV and PS assays. This test and the median and median absolute deviance were determined using log-transformed values of fold resistance. The antilog of the median is shown. Significant results ($P < 0.0033$), based on a Bonferroni adjustment for 15 comparisons, are in bold. One hundred and forty three and 163 mutant protease isolates were tested by ≥1 PI by AV and PS assays, respectively. One hundred and eleven and 79 mutant RT isolates were tested by ≥1 RT inhibitor by AV and PS assays, respectively.

ABC, abacavir; APV, amprenavir; AV, Antivirogram assay; D4T, stavudine; DDC, zalcitabine; DDI, didanosine; DLV, delavirdine; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; ND, not done; NFV, nelfinavir; PS, PhenoSense assay; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; ZDV, zidovudine.

biologic cutoff of both assays for nelfinavir, ritonavir, and saquinavir, resulting in nearly even distributions of isolates above and below the biologic cutoff. Nearly all isolates with I54V + V82A + L90M ± M46I had reduced susceptibility to all PIs.

The biologic cutoffs for the NNRTIs were between 2.73 and 6.89 for the PhenoSense assay and between 4.43 and 12.04 for the Antivirogram assay. Only 3 patterns of RT mutations with NNRTI resistance mutations were tested at least 3 times by both assays (K103N + M184V, K103N, and Y181C), and no significant differences in the sensitivity of the 2 assays for the detection of resistance to isolates with these patterns were found.

DISCUSSION

This study shows that the PhenoSense assay is superior to the Antivirogram assay in detecting resistance to NRTIs with a narrow in vitro dynamic susceptibility range, at least in part because the increased precision of this assay leads to better separation of the susceptibility results of wild-type and mutant isolates. This separation is particularly important for the NRTIs abacavir, didanosine, and stavudine, because low levels of phenotypic resistance to these drugs are clinically significant, particularly in isolates containing multiple thymidine analog mutations.[6–9] We did not notice significant differences between the assays in detecting resistance to PIs, to NNRTIs, or to zidovudine and lamivudine—NRTIs with a wide dynamic susceptibility range.

The Antivirogram and PhenoSense assays each use polymerase chain reaction (PCR) to amplify from patient plasma the entire protease, much of RT (1–400 for the Antivirogram assay and 1–311 for the PhenoSense assay) and the 3′ part of *gag*, including the p7/p1 and p1/p6 cleavage sites.[10–12] The amplified material is incorporated into a *pol*-deleted recombinant virus by ligation (PhenoSense assay) or homologous recombination (Antivirogram assay). A standardized virus inoculum is then used to infect 293 (PhenoSense assay) or MT4 (Antivirogram assay) cell lines, and virus replication is measured in a range of drug concentrations. In the PhenoSense assay, virus replication is monitored by a luciferase gene cassette that emits light in proportion to the number of virions after 1 round of HIV-1 replication. The Antivirogram assay measures the intensity of a tetrazolium dye

TABLE 3. Drug Resistance Prediction Using Biologic Cutoffs for the Most Common Patterns of NRTI Resistance Mutations: Comparison of the Antivirogram and PhenoSense Assays

Pattern of NRTI Resistance Mutations	Proportion of Isolates With Fold Resistance Above the Biologic Cutoff (No. Isolates Above the Cutoff/No. Isolates Tested)														
	3TC			ABC			D4T			DDI			ZDV		
	AV (UL: 2.88)	PS (UL: 1.53)	*P*	AV (UL: 2.75)	PS (UL: 1.35)	*P*	AV (UL: 3.31)	PS (UL: 1.32)	*P*	AV (UL: 3.80)	PS (UL: 1.32)	*P*	AV (UL: 3.03)	PS (UL: 1.87)	*P*
M184V	55/55	18/18	NS	**8/41**	**20/20**	**5E-10**	0/54	0/20	NS	**3/53**	**15/18**	**6E-10**	3/55	0/18	NS
M41L, M184V, T215Y	10/10	12/12	NS	**1/7**	**14/14**	**1E-04**	**0/10**	**9/12**	**5E-04**	**1/10**	**9/12**	**4E-03**	8/10	10/12	NS
M41L, M184V, L210W, T215Y	8/8	7/7	NS	**1/4**	**8/8**	**2E-02**	**0/7**	**6/7**	**5E-03**	**0/7**	**6/7**	**5E-03**	6/8	7/7	NS

The biologic cutoffs of fold resistance for each drug (UL) were derived from the distribution of results obtained testing isolates lacking drug resistance mutations and were defined as the geometric mean results plus 2 standard deviations calculated using the log-transformed levels of fold resistance. The biologic cutoffs are similar to those previously published for the AV[3] and PS[4] assays. The Fisher exact test was used to compare the proportion of isolates with fold resistance above the biologic cutoff for the AV and PS assays. Statistically significant results ($P < 0.05$) are shown in bold.

ABC, abacavir; AV, Antivirogram assay; D4T, stavudine; DDI, didanosine; PS, PhenoSense assay; UL, upper limit; ZDV, zidovudine.

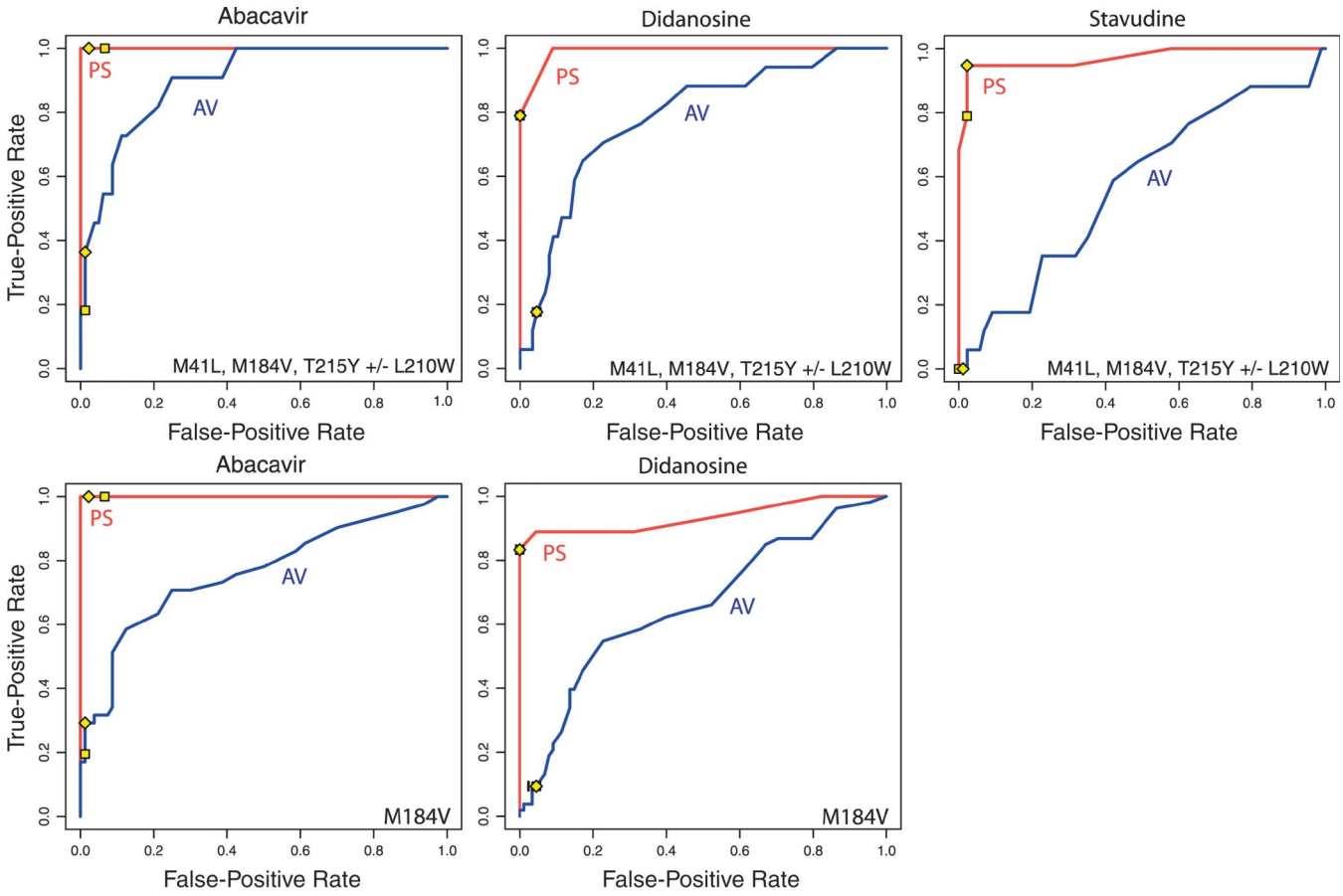

FIGURE 1. Receiver operator characteristic (ROC) curves showing the sensitivity of the Antivirogram (AV) and PhenoSense (PS) assays at detecting resistance to isolates containing the mutations M41L + M184V + T215Y (±L210W) and M184V. The effect of different cutoffs on sensitivity is shown on the *y*-axes, and the false-positive rate (1 − specificity, based on susceptibility data on isolates lacking drug resistance mutations) is shown on the *x*-axes. The sensitivity and specificity of the AV and PS assays are indicated by blue and red curves, respectively. Yellow squares indicate the cutoff used in Table 3 (geometric mean + 2 standard deviations), yellow circles (which are always at the same place on the curves as the squares) indicate the cutoffs from published reports,[3,4] and yellow diamonds indicate the 98th percentile observed in the wild-type isolates in this study. Tests of differences between the areas under the empiric ROC curves showed that the PS assay was significantly more sensitive than the AV assay for abacavir ($P = 0.02$), didanosine ($P < 0.001$), and stavudine ($P < 0.001$) for M41L + M184V + T215Y ± L210W and for abacavir ($P < 0.001$) and didanosine ($P < 0.001$) for M184V alone.[5]

that produces color when reduced by cellular mitochondrial enzymes in proportion to the number of viable cells present after several cycles of HIV-1 replication.

Because the Antivirogram and PhenoSense methods are proprietary, it is only possible to speculate on the basis for the greater precision of the PhenoSense assay. One possible explanation is that the light resulting from the luciferase cassette after a single round of replication is directly proportional to the amount of resistant virus, whereas the color produced by the tetrazolium dye is a less direct measure of resistant virus because it results from decreased cell viability, a downstream consequence of several rounds of virus replication. A second possible explanation is that the virus inoculum for the PhenoSense assay is stoichiometrically controlled at the molecular level by ligating the patient amplicon directly into the test vector in a cell-free reaction rather than by homologous recombination, which occurs at an unpredictable rate after cotransfection into cells of the patient amplicon and test vector. Finally, it is also possible that the different cell lines used in the 2 assays triphosphorylate the NRTI prodrugs at different rates or contain different levels of competing deoxynucleoside triphosphates.

One previous study compared the results of the Antivirogram and PhenoSense assays on 38 clinical isolates, of which a third contained drug resistance mutations,[13] but the small number of mutant samples tested made it impossible to assess the precision and sensitivity of each assay accurately. Our study is limited by the fact that different isolates were tested by each assay. Although isolates were matched for known drug resistance mutations, they differed at polymorphic residues and may have had other unrecognized differences. Because the isolates tested by both assays had similar numbers of mutations at polymorphic residues and because of the magnitude of the difference between the assays for detecting NRTI resistance, it is improbable that the differences between the assays can be explained solely by imbalances at polymorphic sites or other unrecognized differences. Nonetheless, sufficiently powered studies comparing drug susceptibility results on large numbers of identical isolates from cryopreserved plasma samples are needed to provide a reliable ongoing assessment of the performance of these and newer susceptibility assays (eg, Phenoscript).

ACKNOWLEDGMENT

The authors thank Andrew Zolopa for critical review of the manuscript.

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