Standardized Peripheral Blood Mononuclear Cell Culture Assay for Zidovudine Susceptibility Testing of Clinical Human Immunodeficiency Virus Type 1 Isolates: Effect of Reducing the Numbers of Replicates and Concentrations

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Zidovudine susceptibility was assessed for 525 clinical human immunodeficiency virus type 1 isolates, before and after reducing the number of replicates and zidovudine concentrations in the standardized consensus peripheral blood mononuclear cell culture assay. We conclude that omitting the 0.001 μ M concentration and using duplicate rather than triplicate wells are valid and cost-effective modifications of this expensive assay.

Phenotypic zidovudine resistance in human immunodeficiency virus type 1 (HIV-1) isolates from patients on long-term zidovudine treatment has been shown to independently predict clinical decline (2). However, a limitation of the standardized consensus assay for in vitro drug susceptibility of HIV-1 isolates in peripheral blood mononuclear cells (PBMC) (3, 4) is its high cost. This study formally validates a cost-effective modification to the assay that involves reducing the number of replicate wells and the number of zidovudine concentrations at which viral inhibition is assessed.

Zidovudine susceptibilities were determined on 525 HIV-1 isolates obtained from the National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group (ACTG) trial 116B/117 and from the laboratories of the U.S. Department of Defense (DoD) and the University of Minnesota Medical School. These determinations were made by using the PBMC-based assay validated by the ACTG and DoD (3, 4). For each HIV-1 isolate tested, triplicate quantitative p24 antigen determinations were performed with day 7 culture supernatants obtained from acutely infected PBMC. The zidovudine concentrations tested in triplicate were 0, 0.001, 0.01, 0.1, 1.0, and 5.0 μ M. The zidovudine concentration that inhibits virus replication by 50% (IC_{50}) was estimated by fitting a median-effect equation model (1) to the p24 antigen results. This study assessed the precision of IC_{50} estimation after reducing the number of replicates per concentration from three to two and the number of zidovudine concentrations from six to five. In addition, the classification ability of the modified assay was assessed by using the following two phenotypic resistance classifications:

(i) highly zidovudine resistant or not, as defined by an IC_{50} value ≥ 1.0 μ M or < 1.0 μ M, respectively; and (ii) zidovudine sensitive or not, as defined by an IC_{50} value of ≤ 0.1 μ M or > 0.1 μ M, respectively.

The median-effect equation (1) relates the proportion of inhibition of HIV-1 replication to the zidovudine concentration. Estimation of IC_{50} for each isolate was carried out by fitting the median-effect equation by using the nonlinear least squares procedure in the software package SAS (5). The proportion of virus inhibition (fraction affected) for each replicate was defined as $1 - (\text{p24 antigen level/average p24 antigen level of the } 0 \text{ } \mu\text{M replicates})$. To examine the effect of removing replicates and zidovudine concentrations, IC_{50} values were recalculated after removal of one replicate and selected concentrations. Specifically, for each isolate, every possible subset consisting of two replicates per concentration was constructed, and for each subset the IC_{50} was estimated. This process was repeated five times, removing all data from one zidovudine concentration at a time, with the exception that the 0 μ M concentration was always retained. Thus, for each isolate there were six groups of new IC_{50} estimates, one of which was based on a modified assay having two replicates and six zidovudine concentrations (729 new estimates), and five of which were based on modified assays having two replicates and five zidovudine concentrations (243 new estimates in each group). The following summary measures of the difference between the full data set results and each of the reduced data set results were calculated: (i) proportion of reduced data set IC_{50} estimates that were "correctly" classified as either < 1.0 μ M or ≥ 1.0 μ M, where a classification is "correct" if it is the same as the classification based on the full data set; (ii) proportion of reduced data set IC_{50} estimates that were correctly classified as either ≤ 0.1 μ M or > 0.1 μ M; (iii) median of the ratio of the IC_{50} standard error ($\log_{10}$ scale) for the full data set to that of the reduced data sets; and (iv) average absolute difference be-

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TABLE 1. Phenotypic resistance classification ability with two replicates and removal of one or no zidovudine (ZDV) concentrations in the ACTG/DoD consensus PBMC assay

Classification and ZDV concn removed (μM)	%		
	Mean correct ^a	With good classification ^b	With poor classification ^b
ZDV sensitive or not (≤ 0.1 or $>0.1 \mu\text{M}$)			
None	96	91	2
0.001	96	90	3
0.01	95	89	4
0.1	90	79	7
1.0	96	90	2
5.0	91	81	6
High-level ZDV resistant or not (≥ 1.0 or $<1.0 \mu\text{M}$)			
None	97	93	1
0.001	97	93	1
0.01	97	93	1
0.1	96	92	3
1.0	94	88	6
5.0	92	83	6

^a Mean percentage of reduced data sets with the same classification as full data set.

^b Percentage of isolates for which the proportion of reduced data sets with the same classification as the full data set was ≥ 0.8 (good) and ≤ 0.5 (poor).

tween reduced and full data set IC_{50} estimates ($\log_{10}$ scale). Results based on summary measures i and ii are given in Table 1, whereas results based on measures iii and iv are given in Table 2.

Based on the full data set IC_{50} values, 255 (49%) isolates were zidovudine sensitive, 137 (26%) were highly zidovudine resistant, and 133 (25%) were intermediate (IC_{50} between 0.1 and 1.0 μM). As Table 1 shows, a very high percentage of reduced data sets correctly classified IC_{50} as either $\geq 1.0 \mu\text{M}$ or $<1.0 \mu\text{M}$ ($>90\%$ on average), with the same being true for classification as $\leq 0.1 \mu\text{M}$ or $>0.1 \mu\text{M}$. When one replicate was removed, the same level of correct classification was obtained whether or not the 0.001 concentration was included (97% for the $<1.0/\geq 1.0 \mu\text{M}$ classification and 96% for the $\leq 0.1/>0.1 \mu\text{M}$ classification). The magnitude of these values (97 and 96%) suggested that removal of the 0.001 μM concentration, together with removal of one replicate, has little effect on the

ability of the assay to correctly classify an isolate. This was also indicated for the 0.01 μM concentration, as well as the other concentrations to a lesser extent. Similar results were obtained when the percentages of isolates with "good" (≥ 0.8) and "poor" (≤ 0.5) correct classification proportions were considered, as in Table 1. Taken overall, the best results were achieved when one replicate and the 0.001 μM concentration were removed. In this case a large percentage of isolates exhibited a good correct classification proportion (93 and 90% for the two types of phenotypic zidovudine resistance classification), whereas only a small proportion of isolates exhibited a poor correct classification proportion (1 and 3%).

The median ratio of the full data set IC_{50} standard error (on the $\log_{10}$ scale) to that of the reduced data sets was very high (close to 1), when one replicate and the 0.001 μM concentration were removed (median across isolates = 0.97; Table 2). This suggested there was little loss of precision in estimating IC_{50} under such changes to the assay. The loss of precision was greater when one replicate and any of the concentrations 0.01, 0.1, 1.0, or 5.0 μM were removed (0.77 to 0.89; Table 2). The average absolute difference between the IC_{50} estimate based on the full data set and the estimates based on the reduced data sets was generally small, with the median across all isolates ranging from 0.09 $\log_{10}$ to 0.17 $\log_{10}$ for the six modified assays considered (Table 2). Since a difference of the order of 0.1 $\log_{10}$ in IC_{50} estimates would be considered insubstantial from a clinical point of view (assays have a 0.5 $\log_{10}$ run-to-run variability [4]), the results again suggested only a small effect when one replicate and the 0.001 μM concentration were removed (0.09 $\log_{10}$).

In summary, the analyses carried out in this study indicated that measurement of phenotypic zidovudine resistance by the standardized ACTG/DoD consensus assay was not adversely affected by reducing the number of replicate wells to two and the number of zidovudine concentrations from six to five. Removal of the 0.001 μM concentration had the least effect, in terms of both phenotypic resistance group classification and standard error estimation. Thus, it is concluded that the ability to measure phenotypic zidovudine resistance can be adequately retained by adopting a less-expensive modified PBMC assay, having two replicate wells and zidovudine concentrations 0, 0.01, 0.1, 1.0, and 5.0 μM .

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TABLE 2. IC_{50} estimation with two replicates and removal of one or no concentrations, compared to full standardized consensus assay

Concn (μM) removed	Precision ^a	Avg difference ($\log_{10}$) ^b
None	0.94	0.09
0.001	0.97	0.09
0.01	0.89	0.16
0.1	0.86	0.17
1.0	0.82	0.12
5.0	0.77	0.12

^a For each isolate the median was taken of the ratio of the full data set standard error ($\log_{10}$ scale) to the reduced data set standard errors. The median of this quantity over all isolates was then taken.

^b For each isolate the average absolute difference ($\log_{10}$ scale) between the full data set IC_{50} estimate and each of the reduced data set estimates was calculated. The median over all isolates was then taken.

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