JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1247–1249 Vol. 38, No. 3
0095-1137/00/$04.00+0

Use of Calibrated Viral Load Standards for Group M Subtypes of Human Immunodeficiency Virus Type 1 To Assess the Performance of Viral RNA Quantitation Tests

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Received 24 September 1999/Returned for modification 22 November 1999/Accepted 20 December 1999

Optimal management of human immunodeficiency virus type 1 (HIV-1) disease requires accurate quantitation of viral RNA concentrations in plasma. Evidence for increasing geographic intermixing of HIV-1 subtypes makes equivalent quantitation of all subtypes essential. The performances of six quantitative viral RNA tests are described, for the first time, with calibrated viral isolates of diverse subtypes.

The concentration of human immunodeficiency virus type 1 (HIV-1) RNA in plasma is a critical marker for predicting disease progression (19, 20) and for monitoring the efficacy of antiretroviral drug therapy (7, 23). The geographic dispersal of genetically diverse viral subtypes of HIV-1 requires the use of molecular-based assays capable of accurately measuring viral RNA concentrations independent of viral sequence or subtype. HIV-1 has been divided into three divergent phylogenetic groups. Group M is the prevalent group and is subdivided into at least nine subtypes (subtypes A to H and J) (15). HIV-1 groups O and N are both highly divergent and rare (13, 15, 28). First-generation viral RNA quantitation tests were designed for optimal performance with subtype B, which initially predominated in North America and Europe. However, the entry of non-subtype B virus into these areas (1, 5, 6, 16) and the discovery of intersubtype recombinant viruses (18, 26) complicated nucleic acid-based testing (2, 11, 22, 24). Moreover, there is a greater appreciation for the need to evaluate plasma HIV-1 RNA in other regions of the world. The inability of some molecular-based tests to efficiently quantitate viral RNA from non-B subtypes represents a significant problem for clinicians relying on viral RNA concentrations for monitoring antiretroviral drug therapy and for the design of preventive vaccine trials where viral RNA will be used as a clinical endpoint (14, 25).

A previously described panel of 30 HIV-1 isolates of group M subtypes A through G (21) was used to assess the performance of six HIV-1 RNA quantitation tests: Roche Amplicor HIV-1 Monitor test (versions 1.0 and 1.5), Bayer Quantiplex HIV RNA (version 3.0), Digene Hybrid Capture II HIV RNA test (version 1.0), Organon Teknika NucliSens HIV QT test (version 2.0), and Gen-Probe HIV-1 Viral Load Assay (in development). The use of well-characterized viral stocks that are standardized by particle count allows for comparative analysis of the performance of HIV-1 RNA quantitation assays. Improved performance of the Roche Amplicor HIV-1 Monitor test, version 1.5, in the quantitation of A, E, F, and G subtypes was recently demonstrated with this panel (21, 27).

Each member of the HIV-1 subtype panel was diluted into HIV-1-seronegative human plasma to deliver concentrations targeted at 50,000 to 100,000 RNA copies/ml based upon electron microscopic particle counts. Two different dilutions of the HIV-1 panel were used in this analysis because insufficient volume remained of the first dilution to allow for assessment of the Gen-Probe assay. In order to determine that the viral RNA target values were similar between the two dilutions, each viral dilution was retested by both versions of the Roche Amplicor HIV-1 Monitor tests (versions 1.0 and 1.5). Viral RNA values generated demonstrated that both dilutions of the panel gave similar viral RNA concentrations (Table 1). Aliquots of the diluted viral stocks were prepared and stored at −80°C. Vials of each viral isolate were thawed at room temperature for 30 min and vortexed vigorously for 10 s prior to removal for testing. All tests were performed following procedures recommended by the manufacturer of each test.

Viral RNA quantitation results reported in this study represent values generated through singleton testing of each member of the HIV-1 subtype panel. Since viral RNA testing is not performed in replicate in a clinical testing laboratory, all tests were performed in singleton to generate data that reflected the type of data generated by clinical labs. Viral RNA testing was performed by laboratory staff having 2 or more years of experience in the performance of HIV-1 RNA quantitation assays. The laboratory's performance is routinely monitored by participation in Centers for Disease Control and Prevention MEPS or CAP HIV-1 RNA proficiency test panels for Roche Amplicor and Bayer Quantiplex assays.

Comparative quantitation results for both dilutions of the HIV-1 subtype panel members used in this study are summarized in Table 1. Viral RNA values generated for both sets of dilutions did not differ significantly in the Roche Amplicor HIV-1 Monitor test versions 1.0 and 1.5. The mean $\log_{10}$ values for all panel members in version 1.5 of the Roche Amplicor HIV-1 Monitor test were 5.39 ± 0.19 for the first dilution and 5.38 ± 0.25 for the second dilution. A mean value of 4.60 ± 0.84 was calculated for the second preparation of viral dilutions in testing by version 1.0 of the Roche Amplicor HIV-1 Monitor test. These viral RNA values are indistinguishable from those generated when the first viral dilutions of the HIV-1 subtype panel were prepared (21). The high standard deviation observed for the Roche version 1.0 assay is indicative of the greater between-isolate variation for the Roche 1.0 assay

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TABLE 1. Mean $\log_{10}$-transformed RNA values for each HIV-1 subtype as generated by six HIV-1 RNA quantitation tests

HIV subtype (*n*)[a]	Mean $\log_{10}$-transformed RNA value by the following test[b]:						
	Roche 1.0[c]	Roche 1.5	Roche 1.5[c]	Gen-Probe[c]	Bayer 3.0	Digene 1.0	NucliSens 2.0[d]
A (3)	3.15	5.40	5.67	5.72	4.99	5.08	5.11
B (7)	5.34	5.50	5.65	5.56	5.40	5.38	5.40
C (5)	5.29	5.39	5.43	5.36	5.15	5.20	5.33
D (3)	5.15	5.34	5.28	5.39	5.22	5.19	5.52
E (8)	4.03	5.29	5.21	5.35	5.13	4.99	4.79
F (3)	3.90	5.53	5.56	5.74	5.12	5.18	4.60
G (1)	5.14	5.38	5.61	5.97	5.09	5.20	<2.6
Mean (30)	4.60	5.39	5.38	5.50	5.19	5.17	5.04
SD	0.84	0.19	0.25	0.24	0.21	0.22	0.59

[a] Number of HIV isolates in each subtype category.
[b] The HIV-1 RNA quantitation tests used were Roche Amplicor HIV-1 Monitor test (versions 1.0 and 1.5), Bayer Quantiplex HIV RNA (version 3.0), Digene Hybrid Capture II HIV RNA test (version 1.0), Organon Teknika NucliSens HIV QT test (version 2.0), and Gen-Probe HIV-1 Viral Load Assay (in development).
[c] Viral RNA values were generated using the second dilution for the subtype panel.
[d] A value of 2.6 $\log_{10}$, which represents the lower detection limit of the assay, was used for subtype G in the calculation of the mean value for the NucliSens test.

than for the Roche 1.5, Gen-Probe, Bayer Quantiplex, and Digene assays. Standard deviations of 0.08 to 0.20 $\log_{10}$ unit have been reported for three commercial HIV RNA quantitation kits using clinical specimens and spiked plasma, with clinical specimens having greater observed variability (4). In addition, a variability of 0.10 to 0.20 $\log_{10}$ unit can be attributed to operator differences in the Roche Amplicor assay. A difference of 0.5 $\log_{10}$ unit in RNA value is considered to be significant in the performance of viral RNA quantitation assays (17). Table 2 shows the difference in $\log_{10}$-transformed RNA concentrations for the Roche Amplicor HIV-1 Monitor version 1.5 test and all other evaluated tests.

Three assays assessed in this report showed minimal differences between subtypes (Tables 1 and 2). No significant difference in HIV-1 RNA value compared to the Roche version 1.5 value was observed for the majority of the subtype panel members assessed for three of the assays: Gen-Probe HIV-1 viral RNA (27 out of 30), Bayer Quantiplex version 3.0 (28 out of 30), and Digene Hybrid Capture II (27 out of 30). Two panel members (DJ258 [A subtype] and BZ163 [E subtype]) were quantified more efficiently (greater than 0.5 $\log_{10}$ unit) by version 1.5 of the Amplicor HIV-1 Monitor test than by the Bayer Quantiplex test. Two E subtypes (ID12 and ID17) and one F subtype (BZ163) were not quantified as well (less than 0.5 $\log_{10}$ unit) in the Digene Hybrid Capture II test. The highest between-isolate standard deviations were observed for version 1.0 of the Roche Amplicor test and version 2.0 of the Organon Teknika NucliSens test (0.84 and 0.59, respectively).

Similar in performance to version 1.0 of the Roche Amplicor HIV-1 Monitor test, Organon Teknika's NucliSens HIV QT assay did not quantify subtypes E and F as efficiently. This assay, though, generated RNA values that were higher for E and F subtypes than the Roche Amplicor version 1.0 test. The mean $\log_{10}$ differences were 0.49 (E) and 0.92 (F) for the NucliSens test compared to Roche Amplicor version 1.5. Mean $\log_{10}$ differences of 1.18 (E) and 1.82 (F) were observed in the Roche Amplicor version 1.0 compared to version 1.5. The NucliSens test, though, quantified subtype A nearly as well as the Roche Amplicor version 1.5 test. Eight of the panel members had viral RNA values that were significantly lower (greater than 0.5 $\log_{10}$ unit) that those generated by the Roche version 1.5 test. In addition, this assay could not detect the single G subtype isolate. These results are consistent with previously published data (8, 10). A developmental modification of the NucliSens assay using primers from the long terminal repeat and *pol* regions that performs more uniformly across HIV-1 subtypes was recently reported but was not assessed for this analysis (10).

The best comparative concentration values obtained were between version 1.5 of the Roche Amplicor HIV-1 Monitor test and the Gen-Probe Viral Load Assay (mean $\log_{10}$-unit difference of −0.02 [Table 2]). Version 1.5 of the Roche Amplicor HIV-1 Monitor test generates HIV-1 RNA concentrations that are on average 0.20 to 0.27 $\log_{10}$ unit higher than the other assays assessed except for the Gen-Probe assay. Mean $\log_{10}$ RNA values of 5.38 and 5.50 were obtained for the panel by version 1.5 of the Roche Amplicor test and the Gen-Probe test, respectively. In a comparison of viral RNA results generated by the Roche Amplicor version 1.5 and Gen-Probe assays, 18 of the panel members yielded higher RNA concentrations in the Gen-Probe assay, whereas 12 of the members yielded higher RNA concentrations in the Roche Amplicor version 1.5 test. Two panel members (DJ263 [A subtype] and BZ167 [B subtype]) were more efficiently quantified by the Roche Am-

TABLE 2. Mean $\log_{10}$ differences of RNA values for each HIV-1 subtype with respect to the Roche Amplicor Monitor 1.5 test

HIV subtype (*n*)[a]	Mean $\log_{10}$ difference[b]				
	Roche 1.0	Gen-Probe	Bayer 3.0	Digene 1.0	NucliSens 2.0
A (3)	2.58	0.02	0.41	0.32	0.29
B (7)	0.34	0.12	0.09	0.12	0.09
C (5)	0.15	0.07	0.23	0.19	0.06
D (3)	0.13	−0.11	0.11	0.14	−0.19
E (8)	1.18	−0.15	0.16	0.30	0.49
F (3)	1.82	−0.02	0.40	0.35	0.92
G (1)	0.47	−0.36	0.29	0.18	NA[c]
Mean difference (30)	0.89	−0.02	0.20	0.23	0.27
SD	0.87	0.28	0.18	0.18	0.35

[a] Number of HIV isolates in each subtype category.
[b] Mean $\log_{10}$ differences of RNA values for each HIV-1 subtype by HIV-1 RNA quantitation tests compared to the values found by the Roche Amplicor Monitor version 1.5 test. The HIV-1 RNA quantitation tests used were the Roche Amplicor HIV-1 Monitor test (version 1.0), Bayer Quantiplex HIV RNA (version 3.0), Digene Hybrid Capture II HIV RNA test (version 1.0), Organon Teknika NucliSens HIV QT test (version 2.0), and Gen-Probe HIV-1 Viral Load Assay (in development).
[c] NA, not applicable.

plicor version 1.5 test. Panel member NP1465 (E subtype) was more efficiently quantified by the Gen-Probe assay (greater than or equal to 0.5 log unit). The overall higher RNA values generated for this HIV-1 subtype panel by the Gen-Probe test may reflect increased test sensitivity.

The Bayer Quantiplex version 3.0 assay performed well within expected between-isolate variation, with a mean $\log_{10}$ difference value (of the number of RNA copies per milliliter compared to the value by Roche version 1.5 test) of 0.20 ± 0.18 compared to -0.02 ± 0.28 for Gen-Probe. The smaller standard deviation observed for Bayer Quantiplex is favorable for overall performance. Three of the HIV-1 viral RNA tests assessed were designed for high-throughput quantitative performance: Bayer Quantiplex, Digene Hybrid Capture II, and Gen-Probe. Of these assays, Bayer Quantiplex version 3.0 and the Gen-Probe tests were designed for maximal sensitivity and specificity, capable of detecting all HIV-1 subtypes including group O variants (3, 12). The primers used in the Roche version 1.5 test (21) diverge extensively from group O RNA and would not be expected to efficiently amplify these sequences.

We show through the use of a panel of calibrated viral isolates that HIV-1 RNA quantitation tests developed or modified within the past 3 years are capable of quantifying a broader range of HIV-1 subtypes than tests which were developed earlier, when less information was available on the diversity of HIV-1 nucleic acid sequences. This improved performance reflects successful attempts by manufacturers to design HIV-1 viral RNA quantitation tests compatible with increasingly diverse HIV-1 subtypes. However, the process of test refinement must be continued, as the spread of the HIV-1 subtypes results in greater diversity and increased potential for lower efficiency of detection in viral RNA quantitation tests. With global migration of HIV-1 subtypes and recombination between all known HIV-1 group M subtypes, nucleic acid-based assays for the detection and quantitation of HIV-1 must be periodically monitored for their ability to detect and quantify prevalent HIV-1 subtypes.

We thank Digene, Inc., for providing test kits and Maryanne Vahey for manuscript review.

This work was supported in part by cooperative agreement no. DAMD17-93-V-3004 between the U.S. Army Medical Research and Materiel Command and the Henry M. Jackson Foundation for the Advancement of Military Medicine and by a grant from NIH (grant A138518) for J. Overbaugh.

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