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Evaluation of the Cavidi ExaVir Load Assay (Version 3) for Plasma Human Immunodeficiency Virus Type 1 Load Monitoring▽

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We evaluated the new low-cost ExaVir Load (version 3) reverse transcriptase viral load assay against the Roche Cobas Amplicor assay. Results for samples tested using the reverse transcriptase assay correlated well with those obtained with the Roche assay ($r = 0.85$; $n = 202$). The version 3 reverse transcriptase assay shows improved sensitivity compared to the previous version.

Human immunodeficiency virus (HIV) load is the critical measure of response to antiretroviral therapy (12) and is useful for predicting the rate of HIV disease progression in untreated patients (7, 11). However, it is well recognized that the cost of viral load testing using nucleic acid-based tests is generally prohibitive in most resource-limited settings (2, 4, 5, 13).

In this study, we compared the Roche Cobas Amplicor HIV-1 Monitor test (HIV RNA assay) (3), which measures HIV RNA, with the Cavidi ExaVir Load assay (HIV RT assay), version 3, which quantifies reverse transcriptase (RT) enzyme activity (9, 10). Previous evaluation of HIV RT assay version 1 and preliminary retrospective analysis of HIV RT assay version 2 by our group have shown favorable comparisons with the HIV RNA-based assays ($r = 0.89$ and $r = 0.89$, respectively) (6). The aim of the current study was to investigate whether HIV RT assay version 3 could be used as an alternative to the HIV RNA assay for viral load monitoring and to compare the sensitivity of the newly released HIV RT assay (version 3) with that of the previous assay.

Blood was collected into EDTA anticoagulant from adult HIV-seropositive patients attending The Alfred Hospital, with ethics approval and written, informed consent. All assays utilized plasma which had been thawed only once. The HIV RT activity in 1-ml patient plasma samples was determined retrospectively using the ExaVir Load assay, version 2 or version 3 (Cavidi AB, Uppsala, Sweden), according to the manufacturer's instructions (9, 10). Pooled HIV-seronegative plasma was obtained from the National Serology Reference Laboratory, Melbourne, Australia, and was subject to repeat analysis to confirm assay specificity. HIV RNA testing was performed using the Cobas Amplicor HIV-1 Monitor assay, version 1.5, ultrasensitive preparation (Roche Diagnostics) (3) according to the manufacturer's instructions. Data on both variables (HIV RT activity [copies/ml equivalents] and HIV RNA level [copies/ml]) were $\log_{10}$ transformed. Pearson's correlation coefficient (r) was calculated for the correlation between $\log_{10}$ HIV RT activity (copies/ml equivalents) and $\log_{10}$ HIV RNA (copies/ml). Samples within the limits of quantitation specified by the manufacturer for the HIV RNA assay (50 to 100,000 copies/ml) and HIV RT assay version 2 (low limit, 400 copies/ml equivalents; median high limit, 538,000 copies/ml equivalents) and within the limits of detection for HIV RT assay version 3 (median low limit, 170 copies/ml equivalents; median high limit, 451,600 copies/ml equivalents) were used for statistical analysis. The sensitivity of the version 3 assay has been reported by the manufacturer to be 200 copies/ml; further evaluation of the lower quantification limit is currently under way in a multisite analysis.

Two hundred forty-four samples from 166 patients were tested using the HIV RNA assay and the HIV RT assay, version 3. The results for three additional samples from one patient with detectable HIV RNA levels (1,900, 8,900, and 10,200 copies/ml) which were undetectable using the HIV RT assay were excluded from this study. HIV genotype analysis revealed that the patient had subtype B virus and contained only the Y181C drug resistance mutation at the time of sample collection, which is unlikely to have significantly affected the RT assay. Further analysis of the fitness of these samples is under way. Of those included, 202 samples (from 142 patients) gave results within the detectable range for both assays; two results were below the detection limits of both assays. Thirty-six samples were below the detection limit of HIV RT assay version 3 but detectable in the HIV RNA assay (median, 200 HIV RNA copies/ml; range, 50 to 2,500 HIV RNA copies/ml), and four samples were above the detection limit of the HIV RNA assay (>100,000 copies/ml) but detectable with HIV RT assay version 3 (median, 216,975 HIV RT copies/ml equivalents; range, 130,546 to 315,311 HIV RT copies/ml equivalents).

The sensitivity of HIV RT assay version 3 was similar to that of the HIV RNA assay, with 94% ($n = 168$) of all samples with HIV RNA levels of >400 copies/ml being detected, compared with 83% for the previous (version 2) assay ($n = 178$) (Table 1). Furthermore, 98% ($n = 145$) of samples with HIV RNA levels of >1,000 copies/ml were detected using HIV RT assay

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TABLE 1. Comparison of sensitivities of HIV RT assay versions 2 and 3, using plasma samples ($n = 242$) from HIV-positive individuals

HIV RNA level (copies/ml)	No. of samples tested	% of samples detected by HIV RT assay	
		Version 2	Version 3
50–400	64	17	59
401–2,000	57	56	86
2,001–10,000	50	90	96
10,001–50,000	48	100	100
>50,000	23	100	100
>200	199	78	92
>400	178	83	94
>1,000	145	92	98

version 3. The improved sensitivity of HIV RT assay version 3 is comparable to those of other commercially available assays quantifying viral RNA, which have lower detection limits ranging from 40 to 400 copies/ml (1, 13).

An excellent correlation was observed between detectable samples with HIV RT assay version 3 and those with the HIV RNA assay ($r = 0.85$; $P < 0.0001$), with 73% of samples having an agreement of ≤ 0.5 $\log_{10}$ copies/ml and 96% of samples having an agreement of ≤ 1 $\log_{10}$ copies/ml. The differences in correlation and agreement observed between the HIV RT and HIV RNA assays are similar to those observed in several previous studies comparing different commercially available viral load assays quantifying viral RNA (14, 15, 18, 19–21) and with HIV RT assay version 2, using either clinical samples or seronegative plasmas spiked with virus stock (6, 8, 10, 16, 17).

The specificity of HIV RT assay version 3 compared favorably to that of the HIV RNA assay, with 100% specificity ($n = 12$), confirming a previous report on HIV RT assay version 1 from our laboratory (6). The HIV RT assay, which is based on signal amplification, is less prone to contamination than most nucleic acid-based assays, making it ideal for use within resource-limited settings, where PCR suites are generally not available.

The improvements made to HIV RT assay version 3 allow 96 samples/operator to easily be tested in a 5-day working week, which is similar to the 84 samples that can be run by one operator with the HIV RNA assay. The hands-on times per sample for the HIV RT and HIV RNA assays are similar, taking approximately 12 and 11.4 min/sample, respectively. In our laboratory, the cost of the HIV RT assay, including labor and consumables, is approximately one-fifth the price of the HIV RNA assay, providing significant cost savings to the laboratory.

HIV RT assay version 3 compares well to HIV RNA-based viral load testing and demonstrates greater sensitivity and a shorter turnaround time than the previous version. For these reasons, we believe that the HIV RT assay is suitable for viral load monitoring in resource-limited countries.

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