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Stability of Human Immunodeficiency Virus Type 1 RNA in Cerebrospinal Fluid Determined with the AMPLICOR HIV-1 MONITOR Test, Version 1.5 (Ultrasensitive)

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We evaluated the effects of time, temperature, freezing, and thawing on the cerebrospinal fluid viral load by using the Roche AMPLICOR HIV-1 MONITOR test, version 1.5 (ultrasensitive). While a statistically significant decrease from the baseline was observed at 24 h, but not at 6 or 12 h, and with one freeze-thaw cycle, all changes were within the range of intra-assay variability.

The PCR is used to quantify the human immunodeficiency virus type 1 (HIV-1) RNA level or viral load (VL) in plasma. Intra- and interassay variability and the effects of time, temperature, and collection methods on the stability of the plasma VL have been extensively reported (3, 4, 7, 11, 16). A decrease of ≥ 0.5 $\log_{10}$ in the number of copies per milliliter of plasma is considered the minimally significant change when assessing treatment response (10).

PCR is also used to quantify the VL in cerebrospinal fluid (CSF). The CSF VL has been used as a surrogate for the brain VL, in part because CSF can be accessed serially with minimum morbidity. However, the results of studies that attempt to correlate the CSF VL with neurological status or the postmortem brain VL are mixed (1, 2, 5, 6, 9, 12, 15). This is not trivial, as some investigators contend that the CSF VL, not the plasma VL, most strongly correlates with neurological disease and brain viral replication (5, 6, 8). Unlike plasma, there are few published reports of studies that have examined the stability of the CSF VL under various conditions (13; M. Ahmad, K. T. Tashima, A. M. Caliendo, and T. P. Flanigan, Letter, AIDS **13:**1281-1282, 1999), which increases the difficulty of comparing conflicting results.

We measured the intra-assay variability and stability of the HIV-1 VL in archival CSF samples by using the AMPLICOR HIV-1 MONITOR test, version 1.5 (ultrasensitive) (Roche Diagnostics Corporation, Indianapolis, Ind.) sample preparation method to determine if the CSF VL remains stable over time and when samples are exposed to different temperatures and storage conditions or repeated freeze-thaw cycles, as does the plasma VL.

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Sixty-eight samples were obtained from 41 HIV-1-infected adults who signed an Institutional Review Board-approved informed-consent form. Multiple samples from a single subject were collected 6 months apart. Some samples were excluded from individual analyses when insufficient sample amounts remained for the performance of all assays. CSF was obtained by standardized lumbar puncture (14), transported at 25°C to an adjacent laboratory, and centrifuged at 1,500 × *g* for 10 min at 25°C. The supernatant was aliquoted, immediately frozen on dry ice, and stored at −80°C. The AMPLICOR HIV-1 MONITOR test, version 1.5 (ultrasensitive) specimen processing procedure was used in accordance with the manufacturer's instructions for plasma (package insert of AMPLICOR HIV-1 MONITOR test, version 1.5). One operator performed all tests.

To simulate the storage of CSF supernatant at room temperature for up to 1 day before assay, an aliquot was allowed to thaw at a controlled temperature (25°C). After 1 h (baseline) and 6, 12, and 24 h, the CSF was vortexed and 0.5 ml was removed. After extraction of genetic material from samples taken at all time points, the samples were amplified and then serially diluted in a single 96-well plate as part of the Roche AMPLICOR HIV-1 MONITOR test procedure. To simulate the reuse of a single sample for multiple tests over time, we thawed 1 ml of CSF from each lumbar puncture sample and divided it into two 0.5-ml samples, which were then refrozen at −80°C. After 24 h, one of the two specimens was thawed again for 1 h at 25°C and then assayed. The remaining 0.5 ml of CSF remained frozen for 6 months and then was thawed and assayed with a kit from the same lot number. To measure the intra-assay variability of the AMPLICOR HIV-1 MONITOR test, version 1.5 (ultrasensitive) specimen processing procedure, we analyzed paired samples from the same aliquot by

running the assay in duplicate. All individual specimens were measured with the same kit from the same lot.

The RNA values were $\log_{10}$ transformed prior to analysis. To explore the effects of exposure to a temperature of 25°C, within-subjects analysis of variance (ANOVA) was performed with time as the within-subjects factor. Significant ANOVAs were followed by paired *t* tests. A *P* value of ≤ 0.05 was considered statistically significant. Some of the sample sizes were not equal because of inadequate sample volumes for all analyses. For the intra-assay variability experiment, only pairs in which each sample yielded a detectable level of HIV-1 were included. There were 16 pairs in which HIV-1 was nondetectable in both samples and 3 pairs in which one sample yielded a detectable VL and the other did not. In all three of these cases, the VL of the sample in which HIV RNA was detectable was less than 50 copies/ml. For statistical purposes, samples with VLs of less than 50 copies/ml were set at 49. To determine intra-assay variability, 37 samples were assayed in duplicate and the results were $\log_{10}$ transformed and analyzed by paired *t* test. The mean VLs ± standard deviations were 2.7275 ± 0.9863 and 2.7740 ± 0.9406 copies/ml. The intra-assay standard deviation was 0.2047. Correlational analysis revealed a strong intra-assay association between the VLs of the paired samples ($r = 0.98$, $P < 0.001$).

The mean $\log_{10}$ CSF VLs were 3.1596 at 1 h, 3.1362 at 6 h, 3.1144 at 12 h, and 2.9963 at 24 h. Results of within-subjects ANOVA with time (1, 6, 12, and 24 h) as the within-subjects factor revealed an overall significant change in $\log_{10}$-transformed VLs over time [$F(3,90) = 9.02$, $P < 0.001$]. Follow-up paired *t* tests revealed no statistically or clinically significant decline in VL from 1 to 6 h [$t(1,30) = 0.74$, $P = 0.46$] or from 1 to 12 h [$t(1,30) = 1.49$, $P = 0.15$]. There was a statistically significant decline from 1 to 24 h [$t(1,30) = 4.75$, $P = 0.001$].

To determine whether VL changed significantly following either one additional 24-h freeze-thaw cycle or after 6 months of storage at −80°C, a within-subjects repeated-measures ANOVA was performed with time as the within-subjects factor at 1 h (baseline), a 24-h thaw, and a 6-month thaw. The mean $\log_{10}$ CSF VLs were 2.9595 in the baseline sample, 2.8658 in the 24-h sample, and 2.7981 in the 6-month sample. These results revealed a statistically significant change in VL over time [$F(2, 62) = 9.46$, $P < 0.001$]. Follow-up paired *t* tests demonstrated significant declines in VL from the baseline to the 24-h thaw [$t(1,36) = 2.6$, $P = 0.01$], as well as from the baseline to the 6-month thaw [$t(1,33) = 5.17$, $P < 0.001$].

Our results demonstrate that the intra-assay variability of CSF VL testing is similar to that of plasma VL testing (11). There was no statistically significant difference in samples exposed to 25°C for up to 12 h, and despite a statistically significant decline at the 24-h time point, the results were within the range of intra-assay variability. Similarly, while there was a statistically significant decline in VL due to repeated freezing and thawing, this again was within the range of intra-assay variability. This finding is consistent with previously published observations (Ahmad et al., letter) and demonstrates the robust stability of the HIV-1 VL in CSF supernatant when measured by the AMPLICOR HIV-1 MONITOR test, version 1.5 (ultrasensitive) under conditions analogous to those common to multicenter studies.

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