

## Multisite Comparison of Reproducibility and Recovery from the Standard and Ultrasensitive Roche AMPLICOR HIV-1 MONITOR Assays

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**Reproducibility and recovery from the standard and ultrasensitive Roche AMPLICOR HIV-1 MONITOR kits were compared in 19 laboratories. The results were generally similar, but the consistently low level of recovery from the ultrasensitive assay in one laboratory points to the need to include external controls in order to track assay performance.**

Measurements of human immunodeficiency virus (HIV) type 1 (HIV-1) RNA levels in plasma are widely used to monitor responses to antiretroviral therapy. Widespread interest in the quantification of HIV-1 RNA to the lowest possible levels led to the development of the ultrasensitive Roche AMPLICOR HIV-1 MONITOR assay. In this version of the MONITOR assay, sensitivity is increased over that in the standard MONITOR assay by concentrating the virus particles in plasma through centrifugation prior to RNA extraction (2). However, neither the internal quantitation standard nor the external controls that are supplied with the kit are subjected to this concentration step; thus, there is no control for the effects of this step in the current design of the kit. Comparative data on the performance characteristics of the ultrasensitive and standard MONITOR assays are limited to a few studies in individual laboratories (1, 2). A multisite comparison was, therefore, undertaken to assess the impact of the extra centrifugation step on intra-assay variation, interassay variation, and recovery of HIV-1 RNA.

Data were obtained from the HIV RNA Proficiency Testing Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health-sponsored Virology Quality Assessment Program. Under this program, participating laboratories periodically receive panels of coded samples of HIV-1 from a well-characterized, HIV-1 subtype B concentrate spiked into plasma from healthy subjects, usually at five-fold serial dilutions (3). Data from three different panels that were assayed with the standard kit and three others that were assayed with the ultrasensitive kit were included in the analyses. The panels consisted of 16 to 18 coded samples, with 3 to 6 replicates at each of four to five nominal HIV-1 RNA concentrations. The nominal concentrations in the panels assayed with the standard kit ranged from 500 to 312,500 HIV-1 RNA copies/ml, while those in panels assayed with the ultrasensitive kit ranged from 50 to 31,250 HIV-1 RNA copies/ml. These concentrations spanned most of the linear ranges of the two assays. All assays took place between June 1999 and January

2000. To avoid confounding differences between the two versions of the kit with differences among laboratories, the analysis was limited to data from 19 laboratories in which at least five of the six panels were assayed (8 laboratories, five panels each; 11 laboratories, six panels each).

The intra-assay standard deviation (SD) of  $\log_{10}$  HIV-1 RNA concentration for each panel in each laboratory was estimated from the mean square error of a log-log regression of estimated HIV-1 RNA concentration on nominal HIV-1 RNA concentration. The distributions of the SDs for the two versions of the kit were very similar (for the standard kit, 56 SDs [10th percentile, 0.063; median, 0.106, 90th percentile, 0.177]; for the ultrasensitive kit, 50 SDs [10th percentile, 0.058; median, 0.111; 90th percentile, 0.168]). To determine if the SDs from the two assays were correlated, a summary intra-assay SD for each version of the kit in each laboratory was obtained from the mean square error of a log-log regression of estimated HIV-1 RNA concentration on nominal HIV-1 RNA concentration and indicators for the panel. The resulting SDs were positively correlated ( $r = 0.53$ ;  $P = 0.02$ ). Thus, there was some consistency to the intra-assay SDs across the two versions of the kit within laboratories.

Interassay variation was estimated from the expected mean squares from a regression of  $\log_{10}$  estimated HIV-1 RNA concentration on  $\log_{10}$  nominal HIV-1 RNA concentration, indicators for laboratory, indicators for panel within a laboratory, and the interaction of laboratory and  $\log_{10}$  nominal concentration. The interassay SD for the standard kit was  $0.082 \log_{10}$

TABLE 1. Descriptive statistics for the range of median  $\log_{10}$  recovery across panels within 19 laboratories in which both the standard and ultrasensitive HIV-1 MONITOR assays were used

Kit	Range of median $\log_{10}$ recoveries				
	Minimum	Percentiles			Maximum
		25th	50th (Median)	75th	
Standard MONITOR	0.028	0.068	0.129	0.190	0.330
Ultrasensitive MONITOR	0.020	0.070	0.176	0.213	0.356

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TABLE 2. Descriptive statistics for median within-laboratory  $\log_{10}$  recovery for three panels of coded samples that were assayed with the standard HIV-1 MONITOR assay and three that were assayed with the ultrasensitive MONITOR assay

Kit	Panel	No. <sup>a</sup>	Median $\log_{10}$ recovery				
			Minimum	Percentiles			Maximum
				25th	50th (Median)	75th	
Standard MONITOR	017rA	18	-0.127	-0.046	0.069	0.102	0.242
	018rA	19	-0.140	-0.106	-0.087	0.015	0.317
	019rA	19	-0.178	-0.136	-0.032	0.027	0.171
Ultrasensitive MONITOR	005ruA	18	-0.167	-0.051	0.003	0.054	0.326
	006ruA	18	-0.201	-0.061	-0.027	0.021	0.172
	007ruA	14	-0.507	-0.216	-0.136	-0.059	0.158

<sup>a</sup> Number of laboratories that participated in each round of testing.

HIV-1 RNA copies/ml, while the interassay SD for the ultrasensitive kit was 0.096  $\log_{10}$  HIV-1 RNA copies/ml. While these estimates were similar, this analysis could not eliminate the possibility that interassay variation is greater for one version of the kit than the other in a small subset of laboratories. Therefore, a second assessment of interassay variation within each laboratory was obtained by calculating median  $\log_{10}$  recovery for each panel in each laboratory and finding the range of medians for each kit in each laboratory. The distributions of the ranges for the two kits were very similar (Table 1). Furthermore, the ranges for the two versions of the kit were not correlated ( $r = 0.20$ ;  $P = 0.40$ ). There was, therefore, little if any tendency for high or low interassay variation with one version to predict high or low interassay variation with the other.

Finally,  $\log_{10}$  recoveries from the standard and ultrasensitive assays were compared to determine if there were systematic differences in estimated HIV-1 RNA concentrations between the two. Descriptive statistics for the distributions of median  $\log_{10}$  recovery among laboratories show little evidence of differences between the two assays (Table 2). When data were pooled across panels within kits and laboratories, the differences between median  $\log_{10}$  recoveries from the two kits within each laboratory ranged from -0.12 to 0.20  $\log_{10}$  HIV-1 copies/ml (median for the standard assay minus median for the ultrasensitive assay). This range implies that median estimated HIV-1 RNA concentration from the standard MONITOR assay ranged from 76 to 158% of median concentration from the ultrasensitive assay. Differences of 0.20  $\log_{10}$  were obtained in two laboratories. In the other 17 laboratories, differences ranged from -0.12 to 0.10  $\log_{10}$  (76 to 126%). While these values indicate that, on average, the recoveries from the two versions of the kit were very similar, recovery from the ultrasensitive assay was substantially lower than recovery from the standard assay in one laboratory. The median  $\log_{10}$  recovery for panel 007ruA in this laboratory was only -0.507 (31%). Recovery was <50% for 81% of the samples in the panel. This laboratory accounted for the lowest median recovery for each of the three panels that were tested by the ultrasensitive assay (minimums in Table 2). However, recovery from the standard assay in this laboratory was close to the middle of the range across all laboratories. Panels 007ruB and 007ruC, which are recoded versions of panel 007ruA, were also assayed in this laboratory. Median  $\log_{10}$  recoveries were -0.19 (65%) and

-0.26 (55%), respectively. Thus, recovery from the ultrasensitive assay at this site has been consistently low.

It is unlikely that the low recovery was caused by reduced amplification. The optical densities for the internal quantitation standard were similar to those obtained for this panel in other laboratories (data not shown). This could indicate that the problem involves a sample processing step that takes place before the quantitation standard is added, which would point directly to the centrifugation step that is part of the ultrasensitive assay but not the standard assay. The possibility is still under investigation.

In summary, there is no evidence that the additional concentration step in the ultrasensitive assay alters intra-assay or interassay variation. With the exception of one laboratory, the recoveries were also similar for the two versions of the kit. While the results are encouraging, consistently low levels of recovery at one site are grounds for caution. Neither the internal quantitation standard nor the external controls that are supplied with the kit provided any evidence of a problem. However, given the design of the assay, these resources could not detect problems that take place during the concentration step. Only a control or controls that are processed through the entire assay could detect these problems. Laboratory personnel should consider using such a control to periodically monitor the performance of the ultrasensitive assay.

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## REFERENCES

1. **Erali, M., and D. R. Hillyard.** 1999. Evaluation of the ultrasensitive Roche Amplicor HIV-1 Monitor assay for quantitation of human immunodeficiency virus type 1 RNA. *J. Clin. Microbiol.* **37**:792–795.
2. **Sun, R., J. Ku, H. Jayakar, J. C. Kuo, D. Brambilla, S. Herman, M. Rosenstrauss, and J. Spadoro.** 1998. Ultrasensitive reverse transcription-PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma. *J. Clin. Microbiol.* **36**:2964–2969.
3. **Yen-Lieberman, B., D. Brambilla, B. Jackson, J. Bremer, R. Coombs, M. Cronin, S. Herman, D. Katzenstein, S. Leung, H.-J. Lin, P. Palumbo, S. Rasheed, J. Todd, M. Vahey, and P. Reichelderfer.** 1996. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS Clinical Trials Group virology laboratories. *J. Clin. Microbiol.* **34**:2695–2701.