Obtaining Unacceptable Results in Assays for Quantitation of Human Immunodeficiency Virus Type 1 RNA in Plasma Samples

Quantitation of human immunodeficiency virus type 1 (HIV-1) RNA is used for predicting HIV disease progression (2, 10), for determining the need to initiate or change antiretroviral therapy (2), and for evaluating the efficacy of newly developed antiretroviral drugs (12, 15).

A number of assays employing different molecular technologies for measuring plasma HIV-1 RNA levels have been developed in the past few years. Three commercial assays for HIV-1 RNA quantitation are widely used for viral load measurement: (i) the AMPLICOR HIV-1 MONITOR assay (Roche Diagnostic System), based on target amplification by competitive PCR (11); (ii) the Quantiplex HIV RNA assay (Chiron Diagnostics), based on signal amplification by the branched-DNA assay (17); and (iii) the NucliSens HIV-1 RNA QT assay (Organon Teknika), based on quantitative nucleic acid sequence-based amplification (18).

Several comparative studies of performance characteristics such as assay sensitivity, reproducibility, and accuracy of commercially available quantification methods for HIV-1 RNA have been published. Relatively good agreement exists between the results obtained by these assays (3, 4, 6, 9, 14, 16).

Moreover, differences in performance between assays that may affect the results of these may be due to multiple factors, including assay and biological variation as well as specimen handling conditions (1, 5, 7, 8, 13).

However, while results of comparative evaluations of assays for the quantitation of HIV-1 RNA in plasma appear highly sensitive, specific, and reproducible in research laboratories, the same may not be true when they are performed under less stringent conditions by hospitals or commercial laboratories.

On the other hand, in spite of procedural precautions and procedure limitations, sometimes several laboratories obtain unacceptable results for a number of samples. A result is considered unacceptable when the RNA values detected for one or more standards are not correct. Usually, this is due either to the fact that the processed specimens inhibit amplification or to the fact that the RNA is not recovered during specimen extraction. In most instances, reprocessing these samples gives acceptable results. Normally, these unacceptable results are not included in comparative studies performed by research laboratories. The occurrence of unacceptable results is therefore a significant characteristic of these assays.

To determine if assays for quantitation of HIV-1 RNA would give acceptable and unacceptable results in a similar fashion, we compared the efficacies of the NucliSens and AMPLICOR MONITOR assays in obtaining viral load values. Both assays were used to test 1,278 serial plasma samples (648 and 630 samples were tested by the AMPLICOR MONITOR assay and the NucliSens assay, respectively) collected from 426 patients infected with HIV-1. The samples were obtained from populations of similar patients. These patients were admitted to the HIV unit of the Hospital General de Galicia (Santiago de Compostela, Spain) between May 1998 and April 1999. The patients were either being treated with a combination of antiretroviral therapies or receiving no treatment.

We assessed the two assays for quantifying HIV-1 RNA with plasma samples subjected to similar handling conditions, in-

cluding anticoagulant and processing time, storage (temperature and time), and shipping. The two assays were performed in accordance with the manufacturers' recommendations. Given the lack of a universal standard, longitudinal assessment of HIV-1 RNA levels was done by the same assay.

Both assays gave acceptable and unacceptable results in the two populations of samples being tested. The NucliSens assay gave 33 unacceptable results out of 630 samples tested (5.23%), while the AMPLICOR MONITOR assay gave 31 unacceptable results out of 648 samples tested (4.78%) (Table 1). When NucliSens and AMPLICOR MONITOR assays were compared, no significant differences were found between the frequencies of unacceptable results (chi-square test with Yates' correction).

In addition, no cluster in which unacceptable results occurred consecutively was produced. No significant effect of age, gender, HIV status, or viral load on obtaining unacceptable results of HIV-1 RNA was observed either.

This study also showed a high frequency (around 95%) of acceptable results (detectable or undetectable RNA) for the two HIV-1 RNA commercial quantitation assays.

The results of this study show that the AMPLICOR MONITOR assay and the NucliSens assay yielded similar numbers of acceptable and unacceptable results in the 1,278 samples examined and demonstrate that they correlate closely in their ability to obtain acceptable and unacceptable results in quantitative HIV-1 RNA measurement.

In summary, our data support the notion that the two methods for quantification of viral load (NucliSens and AMPLICOR MONITOR assays) agree in terms of prevalence of both acceptable and unacceptable results. However, the percentage of unacceptable results will always be accompanied by the added expense of having to retest those samples. This additional cost increases as the number of unacceptable results increases. Our results clearly indicate that either of the two assays is adequately appropriate for monitoring patients with HIV disease. Nevertheless, despite the use of good laboratory practices, the possibility of obtaining unacceptable results still remains, and it is not possible to identify those runs in which these unacceptable results may be present.

TABLE 1. Comparison of the abilities of AMPLICOR MONITOR and NucliSens assays to quantify HIV-1 RNA in serial plasma samples

Assay	No. of specimens tested	No. of unacceptable results (%)	No. of acceptable results (%)
AMPLICOR MONITOR	648	31 (4.78)	617 (95.22)
NucliSens	630	33 (5.23)	597 (94.77)

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