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Improvement of AMPLICOR Human Immunodeficiency Virus Type 1 Viral Load Test (Version 1.5) by Addition of a Coprecipitant during the RNA Isolation Step

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The effect of the addition of a coprecipitant during the RNA isolation step on the analytical performance of the COBAS AMPLICOR human immunodeficiency virus type 1 (HIV-1) Monitor (version 1.5; Roche) viral load test was tested. Thirty-six specimens including patient samples, positive control samples, and negative control samples were processed in the presence and absence of the Pellet Paint coprecipitant. Specimens processed without the coprecipitant had lower RNA yields, as evidenced by a lower signal for the quantitation standard (QS). In addition, the results for all samples processed with the coprecipitant were acceptable on the basis of the optical density (OD) reading for the QS, whereas the result for one specimen processed without the coprecipitant was unacceptable on the basis of the OD reading for the QS, which required the assay to be repeated. Furthermore, the use of the coprecipitant improved the overall precision of the assay.

The COBAS AMPLICOR human immunodeficiency virus type 1 (HIV-1) Monitor Test (version 1.5; Roche) is a reverse transcription-PCR (RT-PCR)-based assay that is commonly used to measure HIV viral loads (2). In the first step of the assay, a lysis solution is added to the plasma sample and the RNA liberated from viral particles is isolated by a standard ethanol precipitation procedure. Biotinylated primers specific for the *gag* gene of HIV are then used to quantify the viral load by RT-PCR. A quantitation standard (QS), which is a short synthetic RNA transcript that contains complementary *gag* primer sequences, is added to each sample during the lysis step and serves as an internal control. The QS is useful for checking for problems related to poor RNA extraction or poor PCR amplification. It is also used to convert the measurements for the RT-PCR products into the absolute numbers of viral RNA copies per milliliter of plasma. The *gag* and QS RT-PCR products are quantified colorimetrically after differential hybridization to an immobilized individual capture probe, with streptavidin-peroxidase, which binds to the biotin incorporated into the PCR primers.

We have observed that samples must occasionally be reanalyzed because of a weak signal from the RT-PCR of the QS internal control, particularly with new operators of the Roche HIV-1 viral load assay. Because only trace amounts of free RNA are circulating in plasma, typically no RNA pellet is visible after the ethanol precipitation step. We hypothesized that samples with low RT-PCR signals for the QS are due to the poor recovery of RNA because of accidental aspiration of the nonvisible RNA pellet. We therefore tested whether the addition of a coprecipitant, Pellet Paint (Novagen Inc., Madison, Wis.), would improve the rate of recovery of RNA during the RNA isolation step and thus reduce the number of samples that need to be retested. Pellet Paint is a pink dye-labeled polymer that improves the efficiency of RNA precipitation even when the RNA concentrations are very low (2 ng/ml) (1), and it makes the RNA readily visible after precipitation. According to the manufacturer, Pellet Paint does not interfere with various molecular biology techniques, such as RT-PCR (1). The use of Pellet Paint has recently been shown to enhance the sensitivity and reproducibility of a qualitative RT-PCR test for enterovirus (3).

HIV-1 viral load tests (COBAS AMPLICOR HIV-1 Monitor Test, version 1.5; Roche) were performed with samples from HIV-positive patients before and after the addition of 2 μl of Pellet Paint per sample. Figure 1 shows the results of the RT-PCR assay for the QS. Routinely, two dilutions (dilution 1 [1:1] and dilution 2 [1:9]) of the QS RT-PCR products are analyzed by the Roche assay. Specimens with optical density (OD) readings for the QS below 0.15 are invalid for calculation of the HIV viral load because it indicates that there is a problem either with the RNA isolation step or with the PCR amplification. The OD readings for 5 of the 38 samples tested without Pellet Paint were less than 0.15 when dilution 2 was analyzed, and the OD reading of 1 sample was less than 0.15 when dilution 1 was analyzed. In contrast, both dilutions of all of the samples processed in the presence of Pellet Paint had OD readings greater than 0.15. Furthermore, on the basis of the mean OD reading, more RT-PCR product was obtained for the samples treated with Pellet Paint. The average OD readings for dilution 2 were 0.44 ± 0.25 without Pellet Paint and 0.57 ± 0.15 with Pellet Paint ($P < 0.01$). The amount of PCR product was also less variable for the samples treated with Pellet Paint, as evidenced by the smaller range of values (Fig. 1). On the basis of the reported properties of Pellet Paint (1), the most likely mechanism for the higher OD values for the QS is that the addition of Pellet Paint increased the amount of RNA recovered from the samples.

The results of quantitation of the HIV viral load are shown in Fig. 2 for samples processed with and without Pellet Paint.

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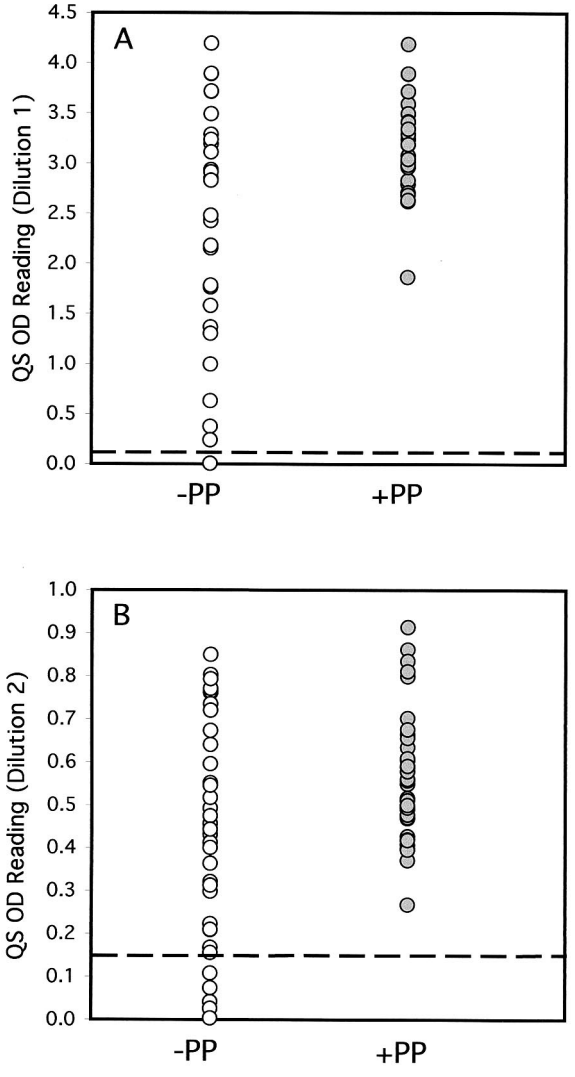

FIG. 1. Scatter plot of OD readings for the QS in the absence (−PP) and presence (+PP) of Pellet Paint. Thirty-six specimens were assayed in three separate runs. The dotted line represents the lower acceptable limit of the OD reading for the QS. OD readings for dilutions 1 (A) and 2 (B) of the QS are shown.

Overall, the results of the two tests showed close agreement, which indicates that the addition of Pellet Paint did not interfere with the RT-PCR measurement of HIV loads. In addition, no significant changes in the results for the positive and negative controls were observed when they were tested in the presence and absence of Pellet Paint.

In order to examine the effect of the addition of Pellet Paint on intra-assay precision, a plasma pool containing approximately 2,000 copies of HIV per ml was analyzed in replicate aliquots with (n = 10) and without (n = 10) Pellet Paint. For analysis of the QS (OD reading of dilution 2), a coefficient of variation (CV) of 51% was obtained without Pellet Paint and a CV of 24% was obtained with Pellet Paint. For the HIV viral load determination, a CV of 61% was obtained without Pellet Paint and a CV of 29% was obtained with Pellet Paint. When the HIV load was expressed as log units, the CVs were 6.3% without Pellet Paint and 4.0% with Pellet Paint. Although the use of the QS in the calculation of the HIV viral load helps control for any variability from the RNA extraction step, it appears that the addition of a coprecipitant further improves the precision of the assay, perhaps by improving the yield and reproducibility of the RNA extraction.

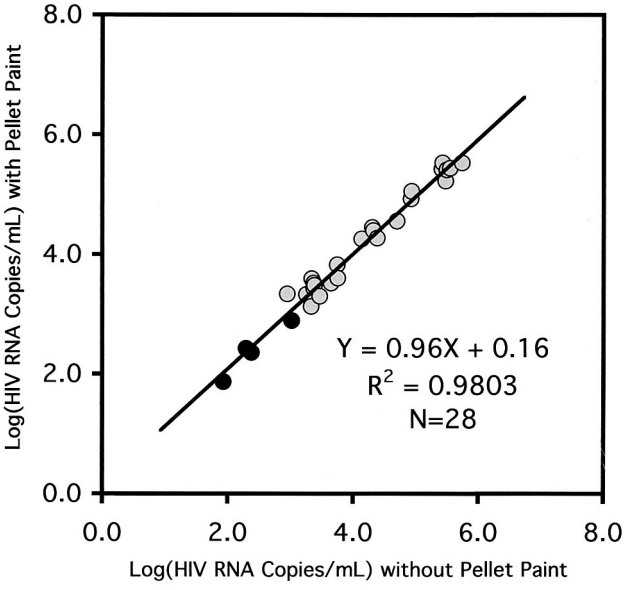

FIG. 2. Correlation of AMPLICOR HIV-1 viral load test results with and without Pellet Paint. Twenty-eight specimens including patient samples and positive control samples were assayed for HIV viral load (24 specimens were assayed by standard assays [shaded circles]; 4 specimens were assayed by ultrasensitive assays [filled circles]). The results are graphed by use of the log of the HIV copy number (number of HIV RNA copies per milliliter).

It is relatively easy to add a coprecipitant to the AMPLICOR HIV-1 viral load assay, and the addition of a coprecipitant does not significantly add to the cost of the test. It costs less than 1% relative to the cost of the AMPLICOR HIV-1 viral load test. Overall, in fact, use of the coprecipitant should result in cost savings because, depending on the experience of the users, it significantly reduces the 5 to 15% retesting rate due to the better recovery of small amounts of RNA.

In conclusion, the two main advantages for the use of the coprecipitant is that it decreases the frequency of sample retesting because of problems associated with low readings for the QS and it improves the overall precision of the AMPLICOR HIV-1 viral load assay.

REFERENCES

1. **McCormick, M.** 1995. Pellet Paint: a visible nucleic acid carrer for efficient, error-free precipitation. Innovations **4:**10–11.
2. **Sun, R., J. Ku, H. Jayakar, J. C. Kuo, D. Brambilla, S. Herman, M. Rosenstraus, 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. **Taggart, E. W., C. L. Byington, D. R. Hillyard, J. E. Robison, and K. C. Carroll.** 1998. Enhancement of the AMPLICOR enterovirus PCR test with a coprecipitant. J. Clin. Microbiol. **36:**3408–3409.