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Affordable, Abbreviated Roche Monitor Assay for Quantification of Human Immunodeficiency Virus Type 1 RNA in Plasma

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The cost for the Roche Monitor assay kit can be reduced 50% by using only the 1:1, 1:25, and 1:625 human immunodeficiency virus (HIV) and the 1:1 quantitation standard dilutions. This abbreviated test applied to 1,774 mostly African samples had results nearly identical to those obtained following the package insert instructions. To make this approach feasible, Roche would have to provide additional lysis buffer and master mix.

With the introduction of generic anti-human immunodeficiency virus (HIV) drugs to resource-poor countries through a variety of national and international programs, antiretroviral therapy (ART) is gradually becoming more affordable. These ART programs use recently developed inexpensive, technologically simple rapid antibody tests to identify infected individuals but rely largely on clinical signs and symptoms to determine when to start and change treatments (5).

In North America and Europe, patients on ART are typically evaluated periodically for HIV disease status and response to therapy using CD4 cell counts and viral load measurements. Quantitation of viral load is usually done by one of three Food and Drug Administration-licensed assays—the Roche Amplicor Monitor (version 1.5), bioMerieux NucliSens QT, and Bayer Versant assays. All of these assays are expensive, with costs ranging from $70 to $200 in the United States. In addition, these assays are technically complicated and require expensive equipment and are best suited for reference type laboratories in resource-poor countries.

Since some laboratories in resource-poor countries have recently been improved with new space, new equipment, and trained technologists, the Roche Monitor assay has become increasingly available in many developing countries, especially in the larger cities. However, despite the recent price reduction by the manufacturer, this assay is still too expensive for many individuals or programs to afford.

The Roche Monitor assay involves HIV RNA isolation, amplification, and detection of amplified products (2). In the manual assay, detection uses six fivefold serial dilutions of the amplicons. In addition, two dilutions of amplified quantitation standard (QS) are also tested. The amount of HIV RNA is calculated based on the following formula:

$$\left[\frac{(\text{OD of the HIV wells}) \times (\text{the dilution factor})}{(\text{OD of the QS}) \times (\text{the dilution factor})}\right] \times (\text{the input QS factor}) \times (\text{the sample volume factor})$$

where OD represents optical density. The automated COBAS assay currently uses five eightfold dilutions.

We questioned whether three 25-fold dilutions of the amplicons would provide similar results. If this were the case, one could test twice as many specimens with the same amount of reagents, thus cutting the kit costs for this HIV RNA assay in half.

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HIV RNA results from 1,575 pregnant women enrolled in a trial of prevention of mother-to-child transmission in Blantyre, Malawi (3, 4), were obtained using the Roche Monitor assay, version 1.5, following the package insert instructions for manual detection. Retrospectively, viral loads from these samples were recalculated using the ODs from the 1:1, 1:25, and 1:625 HIV dilutions only and from the 1:1 QS OD. To determine whether amplicons could be accurately diluted and detected using a 1:25 dilution scheme, specimens being tested in the laboratory were assayed using the Roche Amplicor Monitor assay, version 1.5, following the manufacturer's instructions in the package insert. We also prospectively detected the amplicons of 105 subtype B specimens from patients being monitored at the University of North Carolina Hospital (original assay performed using the COBAS format) and 94 subtype C specimens from pregnant Malawian women enrolled in a different trial of prevention of mother-to-child transmission (1) (original assay performed using the microwell format) from samples by use of only three HIV dilutions (1:1, 1:25, and 1:625) and the 1:1 QS dilution and compared the results to those obtained when all eight wells were used in detection. Approval was obtained from all required Institutional Review Boards.

Viral load was $\log_{10}$ transformed. Data were analyzed using SAS 8.2 (Cary, NC), descriptive analyses, Spearman's and Pearson's correlations, paired *t* tests, and logistic regression.

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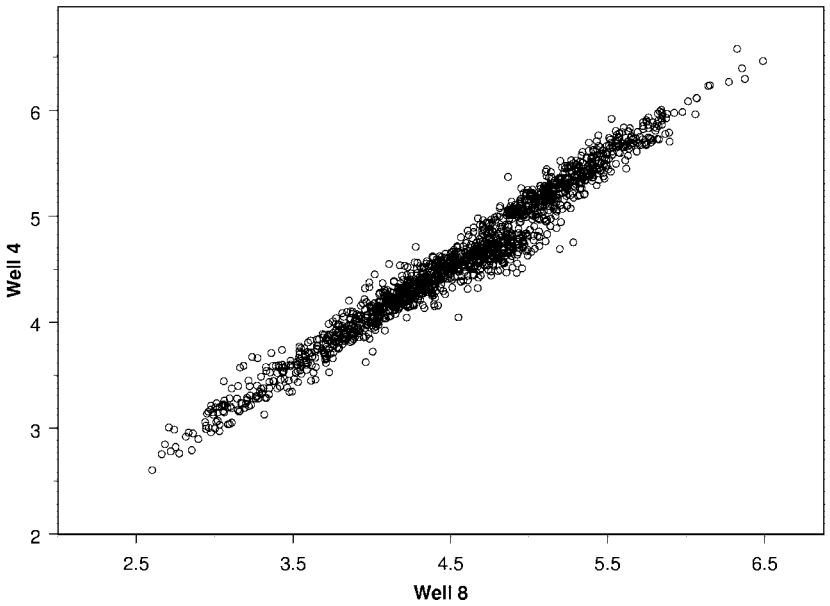

FIG. 1. Correlation between four-well and eight-well assays in the retrospective study. Roche HIV RNA results from 1,575 pregnant women were recalculated using only four wells. Results were indistinguishable. $r = 0.98$.

We found no qualitative or statistical differences between HIV RNA results obtained using all dilutions of the amplicons versus just the 1:1, 1:25, and 1:625 dilutions when selecting the appropriate optical density for the retrospective analysis of viral load in 1,575 pregnant Malawian women infected with subtype C (Fig. 1). The median viral load when all of the wells were used was 4.60 $\log_{10}$ (4.09 to 5.05 $\log_{10}$ interquartile range [IQR]) compared to 4.57 $\log_{10}$ (4.16 to 5.11 $\log_{10}$) using only three dilutions (Pearson's correlation, $r = 0.99$; Spearman's correlation, $r = 0.98$). In multivariate logistic regression mod-

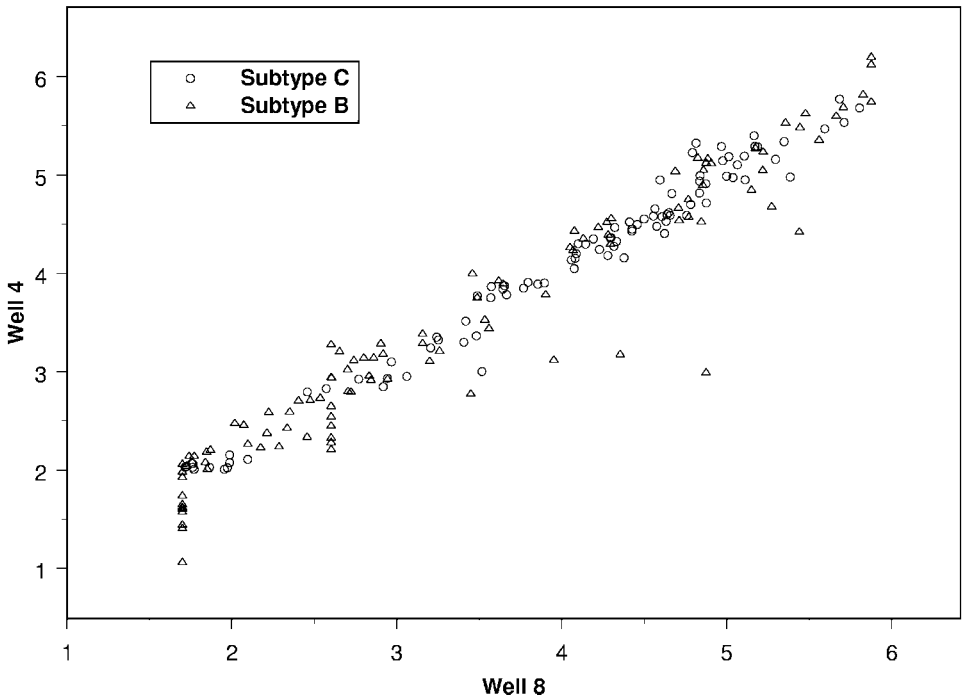

FIG. 2. Correlation between the four-well and eight-well assays in the prospective study. Plasma from 94 subtype C specimens tested in the manual assay (circles) and 105 subtype B specimens tested in the COBAS assay (triangles) were assayed following the package insert instructions and using all eight wells. Then the previously generated amplicons were redetected using only the 1:1, 1:25, and 1:625 HIV dilutions and the 1:1 QS dilution. $r = 0.97$.

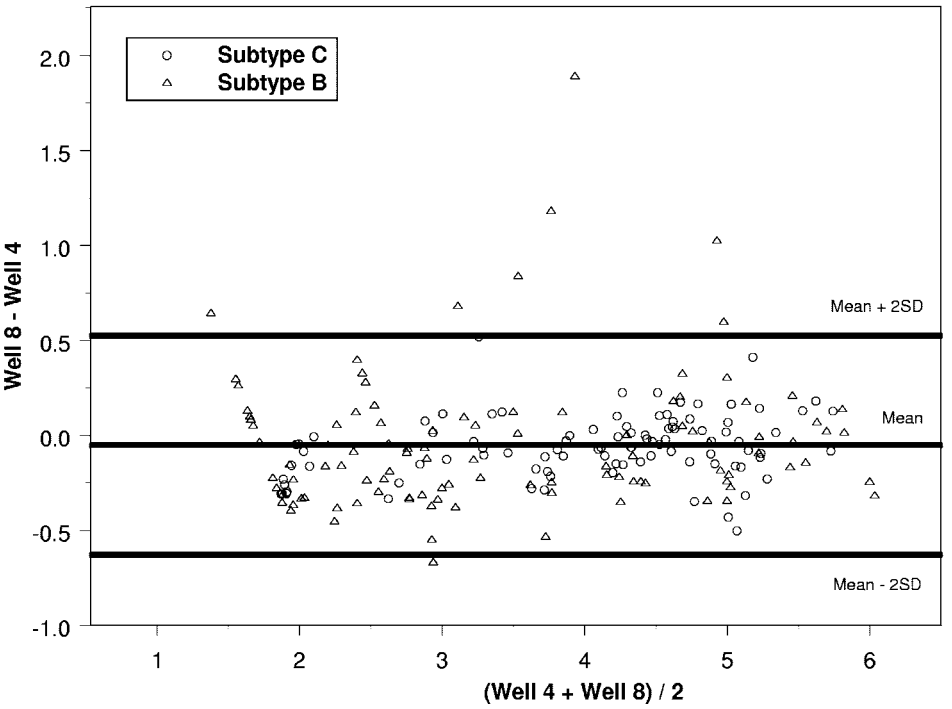

FIG. 3. Bland Altman plot for association of magnitude of deviance between four-well and eight-well tests and underlying mean HIV-1 viral load. Circles indicate subtype C specimens tested in the manual assay, while triangles depict subtype B specimens tested in the COBAS format.

els the four-well and eight-well results predicted subsequent vertical HIV transmission from the mothers to the babies identically (data not shown). In paired *t* tests comparing the log of eight-well results minus the log of four-well results, there was a mean difference of −0.037 (95% confidence interval [CI], −0.044 to −0.030; $P < 0.001$). This means that values from the eight wells were on average about 4% less than those obtained from the four-well analysis—a difference that is clinically irrelevant and well within the coefficient of variation for the assay.

Similar results were observed when 105 subtype B and 94 subtype C specimens being tested in the laboratory were redetected using only three HIV dilutions and one QS dilution; the eight-well data revealed a median viral load of 3.89 $\log_{10}$ (2.60 to 4.78 $\log_{10}$ IQR) compared to four-well data of a median viral load (IQR) of 3.90 $\log_{10}$ (2.77 to 4.71 $\log_{10}$) (Pearson's correlation, $r = 0.97$; Spearman's correlation, $r = 0.97$) (Fig. 2).

There was no difference in results obtained based on original assay format (microwell versus COBAS) or subtype (subtype B versus subtype C). A Bland-Altman analysis confirmed these findings (Fig. 3); virtually all samples fell within 0.5 $\log_{10}$ (2 standard deviations) of the mean, with very few outliers. In paired *t* tests comparing the log of eight-well results minus the log of four-well results, there was a mean difference of −0.058 (95% CI, −0.091 to −0.025; $P = 0.001$) for the 105 subtype C specimens and −0.045 (95% CI, −0.127 to −0.026; $P = 0.21$) for the 94 subtype B specimens. Differences were spread throughout the range of viral loads, and there was no trend to greater discordance with higher or lower viral loads.

Therefore, we have found that using just four wells (three HIV dilutions of 1:1, 1:25, and 1:625 and the 1:1 QS dilution) gave results for HIV RNA plasma viral loads almost identical to those obtained using all eight wells. Consequently, one could test twice as many specimens on a plate, thus reducing the cost of a test by about one-half. The assay worked equally well for subtype B and subtype C specimens and for specimens from treated and untreated men, women, and children. However, lysis buffer and master mix were limiting in quantity and would have to be provided by Roche to make this truly feasible for implementation.

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