A Modified Ultrasensitive Assay to Detect Quantified HIV-1 RNA of Fewer Than 50 Copies per Milliliter

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Abstract

We compared the sensitivity and specificity of versions 1.0 and 1.5 and a modified version 1.5 of the AMPLICOR HIV-1 MONITOR ultrasensitive RNA assay (Roche, Indianapolis, IN) by using a virus stock dilution series and plasma samples from HIV-1–infected and uninfected subjects. The modified assay was linear and consistently positive down to 12 copies per milliliter vs 25 copies per milliliter for the other 2 assays. Versions 1.0, 1.5, and modified 1.5, respectively, detected 9 (23%) of 39, 11 (28%) of 40, and 43 (61%) of 71 replicates of a 4-copy-number standard. Of 44 patient samples with undetectable levels using version 1.0, 32 (73%) had detectable levels on the modified assay, and 5 (25%) of 20 had detectable levels on version 1.5. None of the assays detected HIV-1 RNA in HIV-1 antibody–negative samples. The modified version 1.5 of the RNA assay is more sensitive for detecting HIV-1 RNA in significantly more patients than are versions 1.0 and 1.5.

Materials and Methods

The method for this testing format was adapted largely from Yerly et al.7,8 The version 1.5 Roche AMPLICOR HIV-1 MONITOR ultrasensitive assay was performed according to instructions in the package insert with several modifications: (1) Viral pellets were processed from 1 mL instead of 500 µL of plasma. (2) The high-speed centrifugation step was extended from 60 to 80 minutes using a Heraeus 17RS
centrifuge (Kendro Laboratory Products, Hanau, Germany) at 28,100g. (3) The volume of the internal quantitative standard was reduced from 25 to 6.6 µL per lysis vial. (4) The final concentrated extracted pellet was resuspended in 55 instead of 100 µL of specimen diluent. (5) The substrate incubation time was extended from 10 minutes to a total incubation time of 15 minutes.

The linearity and sensitivity of the modified assay were determined by using replicates of a dilution panel of the virology quality assurance (VQA) standards.9 The VQA dilution series, which consists of HIV-1 subtype B virus, was prepared starting with the 150 copies per milliliter VQA standard (150, 50, 25, 12.5, 6.25, and 3.125 copies per milliliter), with each dilution tested in quadruplicate. The HIV-1 RNA level was determined and compared between versions 1.0 and 1.5 of the AMPLICOR HIV-1 MONITOR ultrasensitive RNA assay and the modified version 1.5 of the ultrasensitive assay. In addition, we tested 39, 40, and 71 replicates of a 4-copy-number standard (a diluted 1500-copies-per-milliliter VQA standard) by using versions 1.0, 1.5, and modified 1.5 of the ultrasensitive assay, respectively, to compare sensitivities. We also tested 40, 37, and 98 replicates of a 10-copy-number standard (a diluted 15000-copies-per-milliliter VQA standard) by versions 1.0, 1.5, and modified 1.5 of the ultrasensitive assay, respectively. For specificity, 40 plasma samples from HIV-1 antibody-negative people were tested by using the modified version 1.5 of the ultrasensitive assay.

For a sensitivity comparison, 35 HIV-1 antibody–positive samples (range, 17-1015,535 copies per milliliter) with at least 2 mL of plasma, in which HIV-1 RNA was detected by version 1.0 of the ultrasensitive assay, were selected randomly and tested with the modified version 1.5 of the ultrasensitive assay. In addition, 44 HIV-1 antibody–positive samples with sufficient remaining plasma were selected from samples with undetectable levels (optical density [OD], <0.2) on version 1.0 of the ultrasensitive assay. These samples then were tested using the modified version 1.5 of the ultrasensitive assay, and 20 of these 44 samples also were tested using version 1.5 of the ultrasensitive assay. All HIV-1 antibody–positive samples were from patients seen in the Johns Hopkins Health Care System, Baltimore, MD, and presumed to have HIV-1 subtype B virus.

Linear regression analysis was performed to show the association among the 3 assays in testing the dilutions of the VQA standard and the association between version 1.0 and modified version 1.5 of the ultrasensitive assay in testing HIV-1 antibody–positive samples. The Wilcoxon matched-pairs signed rank test was used to determine the correlation between results from version 1.0 and modified version 1.5 of the ultrasensitive assay in testing HIV-1 antibody positive samples.

Results

The data obtained by testing the VQA dilution panel on the 3 assay methods correlated closely Figure 1. The HIV-1 RNA levels were similar despite the low number of replicates tested. The modified version 1.5 of the ultrasensitive assay seemed to be linearly to approximately 12 copies per milliliter, with all 4 replicates testing positive down to 12 copies per milliliter and 3 of 4 replicates positive at 6.5 and 3 copies per milliliter. In contrast, versions 1.0 and 1.5 showed all replicates to be positive down to only 25 copies per milliliter. Versions 1.0, 1.5, and modified 1.5, respectively, detected 9 (23%) of 39, 11 (28%) of 40, and 43 (61%) of 71 replicates of the 4-copy-number and 11 (28%) of 40, 17 (46%) of 37, and 88 (90%) of 98 replicates of the 10-copy-number standard.

In addition, 32 (73%) of 44 of patient samples with nondetectable levels using the licensed version 1.0 ultrasensitive assay had detectable levels on the modified version 1.5, and 5 (25%) of 20 had detectable levels on version 1.5 (13/20 [65%] had detectable levels on the modified version 1.5). The OD values of samples tested by version 1.0, which had detectable levels on the modified version 1.5 test (median OD, 0.064; range, 0.039-0.0150 OD), were higher than the values for the samples in which HIV-1 RNA was not detectable by either assay (median OD, 0.048; range, 0.042-0.168 OD, respectively).

All 35 patient samples with detectable HIV-1 RNA by version 1.0 of the ultrasensitive assay had detectable HIV-1 RNA by the modified version 1.5. The values quantitated on the modified version 1.5 ranged from 11 to 149,565 copies per milliliter (vs 17-101,535 copies per milliliter for version 1.0). The log10 values generated by the modified assay were slightly higher than those of version 1.0 (median, 80 vs 53 copies per milliliter, respectively), but the difference was not significant (P = .073). The correlation between the log values of the modified version 1.5 of the ultrasensitive assay and version 1.0 in these positive samples was r = 0.94 Figure 2. In addition, the HIV-1 RNA levels proved to have less than a 0.5-log difference in 31 (89%) of 35 samples and less than a 0.82-log difference in all samples, even at the low end. All of the 40 HIV-1 antibody–negative samples had no detectable HIV-1 RNA by the modified version 1.5 of the ultrasensitive assay.

Discussion

The modified version 1.5 of the ultrasensitive assay was significantly more sensitive than the licensed versions 1.0 and 1.5 for detecting a lower HIV-1 RNA copy number in the VQA dilution series and for more frequently detecting
the 4- and 10-copy VQA standards. Most important, 73% (32/44) and 25% (5/20) of samples with nondetectable levels on version 1.0 had detectable HIV-1 RNA levels on the modified version 1.5 assay and the version 1.5 assay, respectively. This increased detection rate should prove useful for monitoring viral suppression and determining the relative efficacy of different drug regimens for patients in clinical trials who have very low viral copy numbers. It also is reassuring to know that the correlation between the HIV RNA copy numbers detected by the version 1.0 ultrasensitive assay and the modified version 1.5 ultrasensitive assay is very strong, with less than a 0.5-log difference in 89% of subjects (31/35), most of whom had low levels of HIV-1 RNA where variability is greatest. The modified version 1.5 of the ultrasensitive HIV-1 RNA assay seems to be linear down to approximately 12 copies per milliliter, detecting HIV-1 RNA in significantly more patients than versions 1.0 and 1.5 of the ultrasensitive assay licensed in the United States.

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References


