

Comparison of the Sensitivities of the Version 1.5 and Version 1.0 Ultrasensitive Roche AMPLICOR HIV-1 MONITOR Kits at Low Concentrations of Human Immunodeficiency Virus RNA

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The sensitivities of the version 1.5 and 1.0 Roche UltraSensitive AMPLICOR HIV-1 MONITOR tests were compared using panels of coded samples of subtype B human immunodeficiency virus type 1 spiked into plasma at predetermined concentrations. Results indicate that the version 1.5 kit is more sensitive than the version 1.0 kit.

The Roche AMPLICOR HIV-1 MONITOR test version 1.5 was developed in response to substantial underestimation of viral loads in the version 1.0 test, for patients infected with human immunodeficiency virus type 1 (HIV-1) subtypes other than B (1, 3, 4, 5). Most comparisons of the two versions of the kit have focused on differences in estimated viral load when a sample was assayed on both. Comparisons of assay sensitivity have generally been limited to occasional brief comments on the proportions of positive results obtained from parallel assays of clinical samples of various subtypes, although one recent report focuses specifically on sensitivity in assays of low-titer clinical samples (2). Detailed information on the effect of HIV-1 RNA concentration on the difference between the rates of positive results from the two versions is not available. However, it is becoming common in clinical trials and, perhaps, in clinical practice to consider changing treatment when viral loads rise to detectable levels in patients in whom HIV-1 RNA titers have been suppressed below the limit of detection by antiretroviral therapy. Thus, a difference between the sensitivities of the two versions of the kit is potentially important, even for treatment decisions in patients infected with subtypes for which the kits are quantitatively similar. Here, we provide a comparison of the sensitivities of versions 1.5 and 1.0 of the HIV-1 RNA MONITOR kit for subtype B specimens.

Data were obtained from the Virology Quality Assessment (VQA) Program that was established by the Division of AIDS (National Institute of Allergy and Infectious Diseases, National Institutes of Health) to provide quality assurance for clinical trials and other studies of HIV sponsored by the National Institutes of Health (6). As part of this effort, the VQA Laboratory conducts, supports, and collaborates in studies to characterize new assays of HIV-1, such as the sensitivity study that is described here.

The sensitivity comparison was based on coded panels of HIV-1 spiked into HIV-seronegative human plasma at pre-specified HIV-1 RNA concentrations from a well-characterized subtype B stock (6). One panel, which included two HIV-negative samples, eight samples at 25 RNA copies/ml, seven at 50 copies/ml, and seven at 100 copies/ml, was assayed four times on each version. Another panel, which included two HIV-negative samples, five samples at 15 RNA copies/ml, eight samples at 25 copies/ml, seven at 50 copies/ml, and two at 100 copies/ml, was assayed six times on each version. The microwell plate format of the UltraSensitive MONITOR test was used throughout. Three lots of the version 1.0 assay and four of the version 1.5 assay were used. According to the directions in the package insert for the kit, HIV-1 RNA was considered detected (interpreted here to mean that the result was positive) if the optical density (OD) for the undiluted PCR-amplified samples was ≥ 0.20 U, regardless of the estimate of RNA concentration for that sample. An OD of < 0.20 U was considered negative.

The difference in the rate of positive results between versions 1.5 and 1.0 declined with increasing nominal concentration over the range of RNA concentrations tested (Table 1). Statistical tests for individual nominal concentrations are included in the table. A logistic regression of the probability of a positive result on kit version and log-transformed nominal concentration was employed to compare results across nominal concentrations. The results indicate that the version 1.5 test was more sensitive than the version 1.0 test (odds ratio, 3.16; 95% confidence limits, 1.95, 5.12; $P < 0.001$).

In some clinical trials, an assay is considered positive only if an OD of ≥ 0.20 U is obtained and the estimated HIV-1 RNA concentration is ≥ 50 copies/ml, i.e., an assay is negative if all HIV-1 ODs are < 0.20 U or the estimated HIV-1 RNA concentration is < 50 copies/ml. By this approach, larger differences in the rates of positive results were obtained at 25 and 50 copies/ml than at 15 and 100 copies/ml (Table 2). Only one of the differences is even marginally statistically significant, but logistic regression, which has greater statistical power than the

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TABLE 1. Rates of positive results from the version 1.5 and 1.0 UltraSensitive Roche HIV-1 MONITOR tests in assays of coded samples of HIV-1 spiked into plasma when a result was considered positive if the OD for the undiluted PCR-amplified sample was ≥ 0.20

| Nominal concn (copies/ml) | % Positive results (total assays) | | <i>P</i> |
|---------------------------|-----------------------------------|-------------|---------------------|
| | Version 1.0 | Version 1.5 | |
| 0 | 0 (20) | 0 (20) | |
| 15 | 36.7 (30) | 83.3 (30) | <0.001 ^a |
| 25 | 83.8 (80) | 98.8 (80) | <0.001 ^a |
| 50 | 95.7 (70) | 100 (70) | 0.24 ^b |
| 100 | 100 (40) | 100 (40) | |

^a Pearson chi-square.

^b Fisher's exact test.

individual comparisons in this setting, again indicated greater sensitivity for the version 1.5 kit (odds ratio, 1.37; 95% confidence limits, 1.05, 1.81; $P = 0.0221$).

The two versions of the MONITOR kit were also compared using results from the HIV-1 RNA Proficiency Testing Program, which is part of the overall VQA Program (6). Under the Proficiency Testing Program, coded test panels of HIV-1 spiked into human plasma from the stock described above are periodically sent to participating laboratories. Between September 2002 and July 2003, data from proficiency panels were obtained from 5 laboratories in which the version 1.0 kit was used, 9 in which the version 1.5 kit was used, and 15 in which both versions were used, although on different panels. Twelve lots of the version 1.0 kit and at least 13 lots of the version 1.5 kit were included in the analysis. The lot numbers for five runs on the version 1.5 kit were not recorded in one laboratory. The microwell plate format of the version 1.0 kit was used in all 64 assays. The microwell plate format was used for 65 assays on the version 1.5 kit in 22 laboratories, while the COBAS format was used for 10 assays in two other laboratories. Results were

TABLE 2. Rates of positive results from the version 1.5 and 1.0 UltraSensitive Roche HIV-1 MONITOR tests in assays of coded samples of HIV-1 spiked into plasma when a result was considered positive if the OD for the undiluted PCR-amplified sample was ≥ 0.20 and the estimated RNA concentration was ≥ 50 copies/ml

| Nominal concn (copies/ml) | % Positive results (total assays) | | <i>P</i> |
|---------------------------|-----------------------------------|-------------|---------------------|
| | Version 1.0 | Version 1.5 | |
| 0 | 0 (20) | 0 (20) | |
| 15 | 0 (30) | 3.3 (30) | >0.99 ^b |
| 25 | 15.0 (80) | 22.5 (80) | 0.22 ^a |
| 50 | 62.9 (70) | 78.6 (70) | 0.0411 ^a |
| 100 | 100 (40) | 97.5 (40) | >0.99 ^b |

^a Pearson chi-square.

^b Fisher's exact test.

again considered positive if the OD for the undiluted PCR-amplified sample was ≥ 0.20 U. This comparison was limited to samples at 50 copies/ml. Nominal concentrations on all other positive samples were ≥ 500 copies/ml, which was too high to provide meaningful data.

The results from proficiency testing also indicate greater sensitivity on the version 1.5 kit. Fifty-seven of 64 (89.1%) results from the version 1.0 kit were positive, but 74 of 75 (98.7%) results from the version 1.5 kit were positive (Fisher's exact test, $P = 0.0241$). The single negative result from the version 1.5 kit was obtained from the microwell plate format. The estimated HIV-1 RNA concentration was ≥ 50 copies/ml in 32 (50%) assays on the version 1.0 kit and 44 (58.7%) assays on the version 1.5 kit.

Even when attention is confined to subtype B, for which the version 1.0 and 1.5 HIV-1 MONITOR kits are expected to exhibit similar quantitative behavior, positive results at low RNA concentrations are more likely to be obtained from the version 1.5 kit than from the version 1.0 kit. When a result is considered positive only if the estimated RNA concentration is ≥ 50 copies/ml, the difference in rates of positive results from the two versions of the kit is confined to a narrow range of RNA concentrations. Either way, physicians must interpret sudden and unexpected positive results carefully when a laboratory switches from the version 1.0 to the version 1.5 kit.

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