

## Comparison of Versions 1.0 AND 1.5 of the UltraSensitive AMPLICOR HIV-1 MONITOR Test for Subjects with Low Viral Load

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**We compared the performance of two UltraSensitive AMPLICOR HIV-1 MONITOR kits (version 1.5 [v1.5] versus v1.0) by retesting 404 plasma samples with low viral loads (<3,000 copies/ml) with both kits. With 292 samples that initially had <50 copies/ml by the v1.0 kit, the v1.5 assay was more sensitive than the v1.0 assay for samples with human immunodeficiency virus type 1 RNA near the 50-copy/ml cutoff ( $P = 0.0146$ ). Median numbers of copies per milliliter were similar for 112 samples with 50 to 3,000 copies/ml with no difference in sensitivity with a 200-copy/ml cutoff.**

The UltraSensitive AMPLICOR HIV-1 Monitor version 1.5 (v1.5) kit (Roche Diagnostics, Indianapolis, Ind.), which was approved for clinical use by the U.S. Food and Drug Administration in June 2002, has greater sensitivity in detecting certain non-B human immunodeficiency virus type 1 (HIV-1) subtypes compared with the Roche AMPLICOR HIV-1 Monitor v1.0 kit. Prior to the licensure of the v1.5 kit, the majority of HIV-1 viral load assays performed for patient care and clinical trials in the United States were done with the v1.0 kit. The sensitivity of the UltraSensitive AMPLICOR HIV-1 MONITOR v1.0 and v1.5 kits licensed in the United States is approximately 50 RNA copies/ml (5, 6). Since the licensure of the v1.5 kit, anecdotal data have suggested that there might be a difference in the analytical sensitivities of these two kit versions. This possible difference in sensitivity raises concern that a greater proportion of samples with detectable plasma HIV-1 RNA might be observed when the v1.5 kit is used for patients who otherwise had undetectable HIV-1 RNA by the v1.0 kit. This difference in sensitivity could increase the proportion of persons in a clinical trial or in clinical practice who would have a detectable viral load with the new v1.5 test. In addition, a 50- or 200-copy/ml threshold is often used as an endpoint in clinical trials evaluating antiretroviral drugs so that the proportion of persons reaching these endpoints could be different, depending on which kit version was used to test a given population. In order to address this issue, we retested a large number of samples that had relatively low viral loads (undetectable to <3,000 copies/ml) with the v1.0 and v1.5 kits.

The primary objective was to compare the proportions of results that were  $\geq 50$  or  $\geq 200$  copies/ml for plasma samples from persons who initially were found to have <50 or <200

copies/ml by the v1.0 kit when these same samples were retested with the v1.0 and v1.5 HIV-1 RNA assays. The secondary objective was to compare the median numbers of copies per milliliter obtained with the v1.0 and v1.5 HIV-1 RNA assays for plasma samples that were initially found to have 50 to 3,000 copies/ml with the v1.0 kit.

Four hundred four plasma samples were obtained from 216 different HIV-1-infected persons who had been enrolled in a treatment trial between June 2001 and January 2003 (Adult AIDS Clinical Trials Group 5116 study). This trial evaluated reducing the intensity of therapy in patients with viral load suppression on three or four antiretroviral drugs. Plasma samples tested were from persons enrolled in the United States and presumed to be infected with HIV-1 subtype B. These samples had already been tested 1 to 24 months earlier with the v1.0 UltraSensitive assay and initially had either <50, 50 to 199, or  $\geq 200$  copies/ml. Samples were subsequently frozen at  $-70^{\circ}\text{C}$  and not thawed until retested for this study. In both the initial tests and the retests, HIV-1 was considered detected if the optical density (OD) of the first well was  $\geq 0.20$ . In the initial tests with the v1.0 assay, 292 samples (from 146 persons) had <50 copies/ml (172 samples had ODs of <0.20; 120 had ODs of  $\geq 0.20$ ), 83 samples (from 51 persons) had 50 to 199 copies/ml, and 29 samples (from 19 persons) had 200 to 3,000 copies/ml. These samples were retested once with the v1.0 and v1.5 UltraSensitive kits in accordance with the manufacturer's package insert. Only one lot each of the v1.0 and v1.5 kits was used.

We then compared the proportions of persons who had  $\geq 50$  or  $\geq 200$  copies/ml after retesting with both kits. The exact version of McNemar's test was used to compare the difference between proportions.

In retesting the 292 samples with <50 copies/ml, 267 samples were measured as either  $\geq 50$  or <50 copies/ml in both assays (Table 1). Twenty-five samples (8.6%) had discrepant results. Nineteen samples had measured HIV-1 RNA concen-

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TABLE 1. HIV-1 RNA results for persons with initial levels of &lt;50 copies/ml

No. of copies/ml by v1.0	No. of persons with v1.5 level of:		Total
	<50 copies/ml	>50 copies/ml	
<50	264	19 <sup>a</sup>	283
>50	6 <sup>a</sup>	3	9
Total	270	22	292

<sup>a</sup>  $P = 0.0146$ .

trations of  $\geq 50$  copies/ml by the v1.5 assay but <50 copies/ml by the v1.0 assay. In contrast, six samples had measured HIV-1 RNA concentrations of >50 copies/ml by the v1.0 assay but <50 copies/ml by the v1.5 assay ( $P = 0.0146$ ). Sixteen of these 25 samples had ODs of  $\geq 0.20$  on the initial tests with the v1.0 assay. The 13 additional samples detected by the v1.5 assay represent 4.5% of the 292 samples with <50 copies/ml on initial testing.

There were 83 samples with HIV-1 RNA levels initially between 50 and 199 copies/ml that were also retested with both assays. Of these, 36 samples (43.4%) had  $\geq 50$  copies/ml with the v1.5 assay compared with 35 samples (42.2%) with the v1.0 assay ( $P = 0.99$ ). It is interesting that 35 (42%) of these 83 samples had <50 copies/ml and ODs of <0.20 and 13 samples had <50 copies/ml and ODs of >0.2 when retested with the v1.0 kit, even though all had 50 to 200 copies/ml and ODs of  $\geq 0.20$  when initially tested with that version. Conversely, 12 (7.0%) of 172 samples that initially had ODs of <0.20 when tested with the v1.0 kit had detectable virus (ODs of  $\geq 0.20$ ) on retesting with the v1.0 kit.

Combining these results, a total of 375 samples with initial values of <200 copies/ml were retested with both assays. Three hundred sixty-six samples were measured as having either  $\geq 200$  or <200 copies/ml with both assays (Table 2). Nine samples (2.4%) had discrepant results. Seven samples had measured HIV-1 RNA concentrations of  $\geq 200$  copies/ml by the v1.5 assay but <200 copies/ml by the v1.0 assay. In contrast, two samples had a measured HIV-1 RNA concentration of  $\geq 200$  copies/ml by the v1.0 assay but <200 copies/ml by the v1.5 assay ( $P = 0.18$ ).

For 112 samples with initial values of 50 to 3,000 copies/ml, a median estimated HIV-1 RNA concentration of 56 copies/ml was obtained with both the v1.5 and v1.0 kits ( $P = 0.73$ ).

In this comparison of the v1.5 and v1.0 UltraSensitive

TABLE 2. HIV-1 RNA results for persons with initial values of &lt;200 copies/ml

No. of copies/ml by v1.0	No. of persons with v1.5 level of:		Total
	<200 copies/ml	>200 copies/ml	
<200	364	7 <sup>a</sup>	371
>200	2 <sup>a</sup>	2	4
Total	366	9	375

<sup>a</sup>  $P = 0.18$ .

AMPLICOR HIV-1 MONITOR kits, the v1.5 kit had greater analytical sensitivity than the v1.0 kit for samples with HIV-1 RNA levels near the 50-copy/ml lower limit of detection. Therefore, a greater proportion of persons who have undetectable viral loads just below the 50-copy/ml limit will have detectable virus with the v1.5 assay. Many of these samples will give results above the 50-copy/ml limit. This greater sensitivity of the v1.5 kit shown in this study is consistent with results from another report, which showed that in testing replicates of a 10-copy HIV-1 RNA standard, HIV-1 RNA was detected in 46 versus 28% of the replicates tested with the v1.5 and v1.0 kits, respectively (4).

There did not appear to be a significant difference when samples that initially had HIV-1 RNA levels between 50 to 200 copies/ml were tested with the v1.0 kit. Therefore, if a 50-copy/ml endpoint is used to determine failure as opposed to a 200-copy/ml cutoff, a greater proportion of persons may fail with the v1.5 kit, but this difference is highly dependent on the RNA concentration of the sample tested. There did not appear to be a difference between the HIV-1 RNA values obtained with the two kits when samples with 50 to 3,000 copies/ml were tested, as the median numbers of copies per milliliter did not differ. The implication of this finding is that clinical trials that use a 50-copy/ml cutoff as an endpoint and use the v1.0 kit may not want to switch to the v1.5 kit during the trial as the proportion of persons reaching an endpoint might be significantly different.

One interesting finding from this study was that a large proportion of samples (43%) that originally had low-level detectable virus had undetectable virus when the same sample was retested with the same kit. Likewise, 7% of the samples that originally had undetectable virus had low-level detectable virus on retesting of the same sample. These results are not surprising given that the assay is less reproducible near the limit of quantification. These observations are also consistent with studies that show that transient increases in HIV RNA-1 (blips) do not predict virologic failure (within 38 weeks) in patients who have achieved a plasma HIV-1 RNA level of <50 copies/ml on at least one occasion (3). A significant proportion of these transient increases probably represents the variability of the assay at these low concentrations of HIV-1 RNA.

One limitation of our study is that only one lot of each of the assays was used for retesting. However, both lots have been used by several different laboratories that participate in a viral quality assurance program for quantitative HIV-1 RNA proficiency testing (1, 2, 7). In this program, coded quantitative standards of HIV-1 RNA have been tested with these two lots. The data show that the sensitivity of each of these two lots was average for the respective kit versions but may be different from that of the different lots used for initial testing.

Another explanation for the difference in sensitivity between v1.0 and v1.5 may be the discrepant samples having non-B subtypes, which are more likely to be detected by the v1.5 assay. Unfortunately, one cannot subtype these plasma samples, which are from patients with very low viral loads that are far below the threshold (1,000 copies/ml) for subtyping to be performed.

In summary, our data indicate that the v1.5 UltraSensitive AMPLICOR HIV-1 MONITOR kit is more sensitive than the v1.0 kit for samples with HIV-1 RNA levels near the 50-

copy/ml cutoff limit. The effect of this increased sensitivity on endpoints of clinical trials will be dependent on the HIV-1 RNA concentration of the population tested. Likewise, it is likely that clinicians will observe that a greater number of patients with previously undetectable plasma virus will have low-level detectable virus owing to this increased sensitivity.

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