Ability of Two Commercially Available Assays (Abbott RealTime HIV-1 and Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0) To Quantify Low HIV-1 RNA Levels (<1,000 Copies/Milliliter): Comparison with Clinical Samples and NIBSC Working Reagent for Nucleic Acid Testing Assays

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Concordance between molecular assays may be suboptimal at low HIV-1 viremia levels (<1,000 copies/ml); therefore, it may be difficult to define and compare virologic endpoints for successful and failed therapy. We compared two commercial assays (the Abbott RealTime HIV-1 and the Roche Cobas AmpliPrep/TaqMan HIV-1 version 2.0) for their ability to detect and quantify low viral loads. A comparison was performed using 167 residual clinical samples (with values ranging from “not detected” to 1,000 copies/ml, as measured by the Abbott assay) and the National Institute and Biological Standards and Control (NIBSC) HIV-1 RNA working reagent 1 for nucleic acid amplification techniques (NAT) assays (serially diluted to a range from 1 to 1,000 copies/ml). Quantitative results were compared using Lin’s concordance correlation coefficient and a Bland-Altman plot. Concordance results provided by using Lin’s concordance correlation coefficient and a Bland-Altman plot. Concordance with the qualitative results was measured by Cohen’s kappa statistic. With clinical samples, the degree of interassay concordance of the qualitative results at a 40-copies/ml HIV-1 RNA threshold was substantial (κ = 0.762); the correlation among the quantitative results was suboptimal (concordance correlation coefficient, 0.728; P < 0.0001); the mean difference of the values between the Roche and Abbott assays was 0.193 log10 copies/ml. Using the HIV-1 RNA working reagent 1 for NAT assays, the results provided by the Roche assay were, on average, 3 times higher than expected, while the Abbott assay showed high accuracy. The Roche assay was highly sensitive, being able to detect a level as low as 3.5 copies/ml HIV-1 RNA with 95% probability. The performance characteristics of each molecular assay should be taken into account when HIV-1 RNA threshold values for “virologic suppression,” “virologic failure,” “persistent low viral loads,” etc., are defined and indicated in the support of clinical decisions.

The assessment of HIV-1 RNA load in HIV-infected patients is a powerful parameter for monitoring viral suppression and drug efficacy during antiretroviral therapy (ART).

Current international guidelines (GL) and clinical trials define virologic suppression as the achievement and the maintenance of HIV-1 RNA levels <50 copies/ml or to levels below assay limits as the virologic endpoint of successful ART; in fact, below this threshold, patients have the lowest probabilities of morbidity and mortality (1–5). In contrast, the HIV RNA threshold for defining virologic failure (VF) differs across GL. In fact, the World Health Organization GL define VF as a viral load (VL) that is persistently >1,000 copies/ml (from two consecutive VL measurements within a 3-month interval) after ≥6 months of using ART (1). Instead, the British HIV Association (BHIVA), International Antiviral Society (IAS), European AIDS Clinical Society (EACS), and Department of Health and Human Services (DHHS) GL consider VF to be a confirmed VL of >400, ≥200, >50, or >48 copies/ml after suppression, respectively (2–5).

Low HIV-1 viral loads (LVL) (50 to 1,000 copies/ml) represent clinically relevant values for virologic monitoring of HIV-infected patients receiving ART, because they may dictate a need for antiretroviral regimen changes due to an early appreciation of therapy failure (1–5). It is accepted that an increase of HIV-1 RNA VL from a suppressed condition (defined as <50 copies/ml) to levels between 50 and 1,000 copies/ml may predict virologic failure, with a gradation of rebound risk that directly correlates with the degree of viremia. In particular, in patients undergoing suppressive ART, single measurements of HIV-1 RNA between 500 and 1,000 copies/ml seem to be associated with higher rebound risk, with respect to lower values (i.e., <500 copies/ml). To rule out blip or laboratory artifacts, VL increases starting from <500 copies/ml would benefit from confirmation by repeated testing (6, 7).

Furthermore, even HIV-RNA levels <50 copies/ml (residual viremia [RV]) are receiving increased attention, because this level may announce beforehand a viral rebound risk in a manner that is directly proportional to the extent of the RV (8–12).

In this evolving context, it is important to consider the performance features of diagnostic assays used for VL measurements and monitoring. In fact, although most studies have shown that the currently available real-time PCR tests are fairly similar, showing high sensitivity, specificity, reproducibility, as well as good...
correlation in the relatively high range of quantification (13–15),
poor interassay concordance has been well described at LVL (16–
21). This last issue is an important matter of debate regarding
the need to establish a univocal and shared definition of VF and other
virologic definitions (such as virologic suppression, virologic re-
bound, and persistent LVL), because if molecular tests are not in
perfect agreement, it becomes difficult to define univocal virologic
thresholds. Therefore, the performance characteristics of the di-
agnostic systems used to measure VL need to be taken into ac-
count in deciding when to intervene in the management of HIV-1
infection.

Given the great interest in defining VF and LVL in ART-treated
patients for the implications it may have in the clinical setting and
treatment decisions, we analyzed the performance characteristics
of two widely used assays used to measure HIV-1 RNA in order to
clarify the differences between them at LVL. We compared the
Abbott RealTime HIV-1 (referred to as the Abbott assay in this
paper) and Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 ver-
sion 2.0 (referred to as the Roche assay in this paper) assays on
their ability to detect and quantify clinical specimens with LVL
(from “not detected” to 1,000 copies/ml, as established with the
Abbott assay). In addition, the HIV-1 RNA working reagent 1
for NAT assays (HIV-WR1), diluted from 1,053 to 1 copies/ml,
was evaluated with both assays. For VL concentrations of <40
copies/ml, the comparison was conducted in two ways: (i) con-
sidering HIV-1 RNA values from 20 to 40 copies/ml, or to 0 log10
if not detected, in order to align them with the Abbott assay results,
and (ii) using the modified protocol of the Abbott assay (limit of
detection [LOD], 19 copies/ml) (22) in order to compare data for VL of <40 copies/ml with the Roche assay.

MATERIALS AND METHODS
Clinical samples. One hundred sixty-seven clinical plasma samples were
selected among those (n = 11,583) submitted to the Laboratory of Vi-
rology at the National Institute for Infectious Diseases Lazzaro Spallanzani,
Rome, Italy, for routine monitoring of HIV-1 viremia, during the period
between April and October 2011. Routine measurements with the Abbott
assay were performed on fresh plasma samples after overnight storage at
4°C, according to the manufacturer’s instructions. Among all the samples
tested with the Abbott assay, those having HIV-1 RNA levels ranging from
not detected to 1,000 copies/ml and sufficient residual volume were se-
lected and directly tested with the Roche assay, without freezing, in the
same working day.

To avoid HIV-1 RNA decay between the two measurements, all
plasma samples subjected to routine monitoring of HIV-1 viremia were
removed from the Abbott m2000sp automatic extractor immediately after
sampling (about 1 h from the start of the extraction protocol) and were
maintained at 4°C until the end of the amplification procedure and read-
ing of Abbott assay results; overall, the samples remained at 4°C for about
6 h before starting the Roche assay. The elaboration of the results for the
purpose of the study was performed after complete anonymization of the
samples.

HIV-1 RNA assays. Viral load assays were used according to the manu-
facturers’ instructions. The Abbott assay (Abbott RealTime HIV-1; Ab-
bott Molecular, Inc., Des Plaines, IL, USA) was performed on the m2000
sp/r instruments. The stated linear quantification range of the standard
version is 40 to 10,000,000 copies/ml (0.6-ml protocol), and the
reported LOD is 40 copies/ml. For samples with HIV-1 RNA levels of <40 copies/
ml, a modified protocol of Abbott assay, with an LOD of 19 copies/ml, was
used (22). The modified protocol of the Abbott assay was obtained by
introducing some changes to the standard protocol, namely, using a new

calibration curve to cover low HIV-1 RNA concentrations (10 to 1,000
copies/ml), reducing the volume of the internal control, and adopting
open-mode software for quantification. The performance characteristics
of the modified version were previously described, showing high accuracy
and precision (22).

The Roche assay (Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 ver-
sion 2.0, Roche Molecular Systems, Inc., Pleasanton, CA, USA) was per-
formed on the docked configuration of the Cobas/TaqMan 96 instru-
ment. The reported quantification range of the current version, version
2.0, is 20 to 10,000,000 copies/ml, and the declared LOD is 20 copies/ml.

HIV-1 subtype. HIV-1 subtype assignment was retrieved from the
HIV-pol sequences, in the context of a drug resistance mutation pattern
assessment, and was available for 80 of the 167 (47.9%) patients: 73 were
B subtype; 2 were A1; 1 was AG/B, 2 were C, 1 was CRF02/AG, and 1 was
F1. For subtype establishment, the HIV-1 pol sequences were aligned in
BioEdit and compared to reference sequences for the major HIV-1 sub-
types and circular recombinant forms (CRFs), available on the Los
Alamos database (http://www.hiv.lanl.gov). Subtype classification was
confirmed using the REGA (http://www.bioafrica.rega-genotype
/html/subtypinghiv.html) and the COMET (http://comet.retrovirology
.lu/) subtyping tools.

Quality-control material. Multiple replicates of a dilution panel were
prepared from the HIV-WR1 (NIBSC code 96/634-004 [www.nibsc.ac
.uk]). This is a working preparation whose use is intended for monitoring
the performance of diagnostic assays over a period of time. It consists of
HIV-1 subtype B virus, diluted in pooled human plasma samples, with an
assigned value of 3.56 log10 IU/ml, calculated against the 1st International
Standard for HIV-1 RNA (23). For the performance analyses described
here, HIV-WR1 was diluted with HIV-1-negative human plasma (Base-
matrix; Boston Biomedica, Inc., West Bridgewater, MA) to obtain the
concentration indicated in copies/ml, after considering the common con-
version factor of 1 IU as 0.6 copies, according to declarations by Abbott
and Roche. For the comparison of the two assays, five 2-fold dilutions
(from 1,053 down to 66 copies/ml) of HIV-WR1 were prepared, and three
replicates for each dilution were tested. For the comparison with VL val-
ues of <40 copies/ml, eight 2-fold dilutions (from 128 down to 1 copies/
ml) of HIV-WR1 were prepared, and 7 replicates for each dilution were
analyzed.

Data analysis. All VL data were analyzed as log10-transformed values.
In the correlation analysis, the VL data were lined up to 1.59 log10 cop-
ies/ml (39 copies/ml) if detected, <40 copies/ml, or to 0 log10 if not de-
tected. The concordance of qualitative results was measured by the Stuart-
Maxwell test and Cohen’s kappa statistic. The correlation between the
quantitative results was computed as the concordance correlation coef-
ficient (ccc) of the measurements, according to Lin (24). The agreement
between the assays was assessed with a Bland-Altman plot (25). A probit
model was used to describe the response probability of the assays as a
function of HIV-1 RNA concentration (26). Applying the inverse of the
estimated function, we computed the value of the independent variable,
with the relative 95% confidence interval, corresponding to a desired re-
response probability (27). In this way, we also computed the analytical sen-
sitivity (i.e., the minimal concentration of HIV-1 RNA detected with re-
response probability of 95%, or the LOD) of both assays (including the
modified Abbott protocol) using the HIV-WR1 dilutions.

RESULTS
Assay comparison on clinical samples. A comparative evaluation
between the Abbott and Roche assays on their ability to quantify
LVL of HIV-1 RNA was performed using 167 residual clinical
samples, with HIV-1 RNA levels ranging from not detected to
1,000 copies/ml with the Abbott assay. The degree of interassay
concordance of the qualitative results at the 40-copies/ml HIV-1
RNA threshold was substantial (κ = 0.762). The proportion of
samples with an HIV-1 RNA result of not detected was similar
between the two assays (34.7% with Abbott versus 36.5% with

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Roche); the percentage of samples detected above and below 40 copies/ml was similar as well (65.3% with Abbott versus 63.5% with Roche). However, among the samples with detected HIV-1 RNA, the Roche assay provided a significantly higher percentage of samples quantified above 40 copies/ml than did the Abbott assay (42.5% versus 35.9%, \( P = 0.0116 \), Stuart-Maxwell test). Discordant samples between the detected 40 copies/ml and the detected 40 copies/ml in the two assays were 19 (11.4%) and had the following results: all four samples quantified by the Abbott assay had a result of detected 40 copies/ml with the Roche assay; among the 15 samples quantified by the Roche assay, 10 had a result of detected 40 copies/ml (6.0%) and five were not detected (3.0%) with the Abbott assay. The HIV-1 subtype was known for 11 out of 19 (57.8%) discordant samples, and all of them were subtype B (Table 1).

Considering the 56 samples with precisely quantified VL (>40 copies/ml) in both assays, the distribution of values is shown in Fig. 1A. The mean values (± standard deviation [SD]) obtained with the Roche assay (mean ± SD, 2.52 ± 0.53 log\(_{10}\) copies/ml) were 0.193 log\(_{10}\) copies/ml (95% limits of agreement, –0.429, 0.816) higher than the mean ± SD values obtained with the Abbott assay (2.33 ± 0.43 log\(_{10}\) copies/ml), with a Lin’s ccc interassay concordance of 0.728 (\( P < 0.0001 \)) (Fig. 1B). A difference of >0.3 log\(_{10}\) copies/ml was observed in 21 (37.5%) samples (19 with the Roche assay and 2 with the Abbott assay); a difference of >0.5 log\(_{10}\) copies/ml was observed in 9 (16%) samples (8 with the Roche assay and 1 with the Abbott assay) (Fig. 1C). Overall, in more than half of precisely quantified samples (30 [53.6%]), higher levels of quantification were observed with the Roche assay (Fig. 1C).

To see if HIV-1 non-B subtypes affected the comparison between the assays, the analysis was restricted to 73 samples with a known HIV-1 B subtype. Similar and significant differences in the HIV-1 RNA measurements between the Abbott and Roche assays were observed. In fact, the concordance of the qualitative results at the 40-copies/ml HIV-1 RNA threshold concerned 62 (84.92%) of the samples (\( k = 0.684 \)), with 39 (53.42%) samples below and 23 (31.50%) samples above the cutoff for both assays. In addition, considering the 34 subtype B specimens with HIV-1 RNA detected (from detected <40 copies/ml to quantified) with both assays, the mean ± SD VL value obtained with the Roche assay (2.17 ± 0.46 log\(_{10}\) copies/ml) was 0.203 log\(_{10}\) copies/ml (95% limits of agreement, –0.877, 0.471) higher than that obtained with the Abbott assay (1.96 ± 0.36 log\(_{10}\) copies/ml), with a Lin’s ccc interassay concordance of 0.590 (\( P < 0.0001 \)). In six (8.2%) samples, a difference of >0.3 log\(_{10}\) copies/ml was observed, with the Roche assay results being higher; a difference of >0.5 log\(_{10}\) copies/ml was also observed in another six (8.2%) samples (five with higher Roche assay results and one with higher Abbott assay results).

### TABLE 1 Discordant Abbott and Roche assay VL results for the 11 HIV-1 clinical samples known to be subtype B

<table>
<thead>
<tr>
<th>Patient identification</th>
<th>HIV-1 RNA (copies/ml) detected with:</th>
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<tr>
<td></td>
<td>Abbott assay</td>
</tr>
<tr>
<td>1</td>
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<td>20</td>
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<tr>
<td>69</td>
<td>127</td>
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<td>71</td>
<td>63</td>
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[FIG 1] Log\(_{10}\)-transformed VL results of 56 clinical samples given as >1.60 log\(_{10}\) copies/ml (>40 copies/ml) with both Abbott and Roche assays. (A) Dashed line indicates the 1.60-log\(_{10}\) copies/ml (40 copies/ml) cutoff value; solid line shows mean VL. (B) Concordance graph of the observed data, with the linear regression line (solid) and the line of perfect concordance (dashed). (C) Bland-Altman plot of VL values obtained with the 2 assays, with the linear regression line (solid) and the line of perfect agreement (dashed).
The concordance between the Abbott and Roche assays was also examined for HIV-1 RNA cutoffs of >40 copies/ml. The interassay concordance for HIV-1 RNA levels at 50 copies/ml was 89.81% (with 58.68% of the samples below and 31.13% above the threshold with both assays). Increasing the HIV-1 RNA cutoffs to 200 and 400 copies/ml for both the Abbott and Roche assays, the percentages of concordant samples with both assays increased to 92.20% and 95.20%, respectively.

**Assay comparison with the NAT assay reference standard HIV-WR1.** To compare the two assays in their ability to quantify VL, serial dilutions of the HIV-WR1, covering the range of 66 to 1,053 copies/ml, were analyzed. The results show an overestimation of the HIV-WR1 by the Roche assay at all concentrations, while the Abbott results are in line with the expected values (Table 2). Despite a high Pearson’s correlation coefficient between the assays ($r = 0.961$, $P < 0.0001$), the concordance between two assays was very low (Lin’s ccc, 0.498; $P < 0.0001$) due to a considerable distance of the HIV-WR1 measured values from those on the line of the perfect concordance, as shown in Fig. 2A. A Bland-Altman plot of the HIV-WR1 expected and obtained values shows that the data produced by the Roche assay were systematically higher ($0.522 \log_{10}$ copies/ml; 95% limits of agreement, 0.347 to 0.697) than those that were expected (Fig. 2B), while the Abbott assay gave results almost perfectly aligned ($-0.045 \log_{10}$ copies/ml; 95% limits of agreement, −0.217 to 0.128) with the expected values (Fig. 2C).

Next, we focused our attention on a lower range of HIV-1 RNA concentrations. In this case, the comparison was performed using the modified protocol of the Abbott assay (reported LOD, 19 copies/ml) (22), in order to use assays with presumed comparable sensitivities. We first reassessed the actual analytical sensitivity of each of the two assays (i.e., the minimal concentration of HIV-1 RNA detected with response probability of 95%, or the LOD), using 7 replicates of each 2-fold dilution (from 128 to 1 copies/ml) of the HIV-WR1. By probit analysis, the minimal concentration of HIV-1 RNA detected with a probability response of 95% resulted using 7 replicates of each 2-fold dilution (from 128 to 1 copies/ml) (22), in order to use assays with presumed comparable sensitivities.

**Assay comparison at higher HIV-1 RNA thresholds.** In an attempt to better clarify the differences between the VL values given by the two assays at the clinically important VL level of 50 copies/ml, we directly compared the results obtained with the two assays using a subgroup of clinical samples and of HIV-WR1 dilutions whose VL concentrations with the Abbott assay were around 50 copies/ml. The mean HIV RNA values with the Abbott assay were 54 copies/ml for clinical samples (HIV-1 RNA range, 44 to 63 copies/ml; number of samples, 9) and 50 copies/ml for HIV-WR1 dilutions (HIV-1 RNA range, 43 to 53 copies/ml; number of samples, 6), compared to 134 and 168 copies/ml, respectively, obtained with the Roche assay, with 2.48- and 3.36-fold differences, respectively ($P = 0.022$ and <0.001, respectively).

**Table 2.** Results obtained with the Abbott and Roche assays on HIV-WR1 with dilutions from 66 to 1,053 copies/ml.

<table>
<thead>
<tr>
<th>Data type</th>
<th>Abbott assay</th>
<th>Roche assay</th>
</tr>
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<tbody>
<tr>
<td>Nominal values</td>
<td>Log10 copies/ml</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>Copies/ml</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>No. of values</td>
<td>3333333333</td>
</tr>
<tr>
<td></td>
<td>Median (minimum-maximum) (log10 copies/ml)</td>
<td>2.173 (1.590–1.890)</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.1607</td>
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<tr>
<td></td>
<td>Lower 95% CI of mean</td>
<td>2.101 (2.000–2.110)</td>
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<tr>
<td></td>
<td>Upper 95% CI of mean</td>
<td>2.231 (2.210–2.250)</td>
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<tr>
<td></td>
<td>Coefficient of variation (%)</td>
<td>9.06</td>
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<tr>
<td></td>
<td>Mean difference vs nominal values</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>CI, confidence interval</td>
<td>0.022 and &lt;0.001</td>
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</table>

CI, confidence interval.
When restricting the clinical sample comparison to subtype B ($n = 5$), a similar difference was observed (mean, Abbott versus Roche, 53 versus 114 copies/ml; $P = 0.037$).

**DISCUSSION**

In this study, we compared the characteristics of two diagnostic assays widely used for the quantification of HIV-1 RNA in a range of values important for therapeutic decisions, i.e., <1,000 copies/ml. The study was based on both clinical samples and serial dilutions of the HIV-WR1. The results indicate that in the range of 40 to 1,000 copies/ml HIV-1 RNA, the correlation between the two assays is lower than that described at higher concentrations (Lin’s $r$, 0.728, $P < 0.0001$), and poor concordance at the clinically relevant value of 50 copies/ml was observed.

**FIG 2** Log$_{10}$-transformed HIV-RNA results of HIV-WR1, diluted to obtain 1,053 to 66 copies/ml and measured with Abbott and Roche assays (3 replicates/dilution). (A) Concordance graph of the observed data, with the linear regression line (solid) and the line of perfect concordance (dashed). (B) Bland-Altman plot of obtained and expected HIV-RNA values of HIV-WR1 produced by Roche, with the linear regression line (solid) and the line of perfect agreement (dashed). (C) Bland-Altman plot of obtained and expected HIV-RNA values of HIV-WR1 produced by the Abbott assay, with the linear regression line (solid) and the line of perfect agreement (dashed).

**FIG 3** Log$_{10}$-transformed VL results of HIV-WR1, diluted to obtain 128 to 16 copies/ml and measured with modified Abbott and Roche assays (7 replicates/dilution). (A) Concordance graph of HIV-RNA results, with the linear regression line (solid, black) and the line of perfect concordance (dashed, gray). (B) Bland-Altman plot between obtained and expected HIV RNA values obtained with the Roche assay, with the linear regression line (solid, black), the line of perfect agreement (dashed, gray) and the line of mean difference between the assays (solid, gray) along the considered range of HIV-1 RNA values (128 to 16 copies/ml). (C) Bland-Altman plot of HIV RNA values obtained with the modified Abbott assay, with the linear regression line (solid, black), the line of perfect agreement (dashed, gray) and the line of mean difference between the assays (solid, gray) along the considered range of HIV-1 RNA values (128 to 16 copies/ml).
confirmed, both with clinical samples and the HIV-WR1. As both assays have been optimized for the HIV-1 B subtype, as the HIV-WR1 represents a single subtype B sample, and as half of the patients were infected by HIV-1 B subtype (including most of those with discordant results), the HIV-1 B subtype does not seem to be crucial (or the sole factor) for influencing the different performances of these molecular systems (in fact, 11 of 19 patients with discordant results were infected with the HIV-1 B subtype, and 7 patients with HIV-1 non-B subtypes were similarly measured with both assays).

In addition, using the HIV-WR1 preparation, we observed that within the range of the HIV-1 RNA concentrations analyzed here, the Roche assay produced HIV-1 RNA values about 3-fold higher than those obtained with the Abbott assay. On the other hand, the Abbott assay provided HIV-WR1 RNA results that were perfectly aligned with the expected ones, using both the standard protocol (down to 40 copies/ml) and the modified protocol (down to 19 copies/ml) (22). Therefore, it is reasonable that the higher values obtained with the Roche assay may be due to overestimation. However, according to the probit analysis, the Roche assay showed a higher analytical sensitivity, as it was able to detect the presence of HIV-1 RNA at loads well lower than the claimed LOD (20 copies/ml), precisely, until 3.5 copies/ml, which is in agreement with recent data (28).

Overall, our results on the performance characteristics of these two assays at LVL are aligned with similar data previously reported with clinical samples (20, 29–31) and with the 2nd international HIV-1 RNA WHO standard (19). In particular, although there is good agreement between the commonly used diagnostic assays for detecting both HIV-1 B and non-B subtypes along the common range of detection (14, 15, 18, 32–34), the interassay correlation is lower at the lower limit of quantification (16–18, 21, 30, 31, 35). Also, the concordance between the samples with detected or not detected VL is rather low (32–34), with both the clinical samples (11), the 2nd International HIV-1 RNA WHO Standard, and commercial test panels for HIV-1 (16, 19, 20). In line with a recent multicenter comparison study (31), we observed that interassay concordance for a threshold of 200 copies/ml was much higher (92.20%) than that at the threshold of the clinically relevant value of 50 copies/ml (89.81%).

In light of the reported differences in HIV-1 RNA quantification and of the different sensitivities among the existing commercial HIV-1 VL assays, most GL recommend the use of a single method for therapeutic monitoring of individual patients (1–5). Furthermore, several current GL advise not to change ART in HIV-1-infected patients when VL remains at <200 to 1,000 copies/ml (1–5), taking into account the possibility that it may represent a blip (6, 7) and considering also the suboptimal performances and the poor interassay concordance at LVL. On the other hand, while on ART, a careful evaluation of persistent HIV-1 RNA in the range of 200 to 1,000 copies/ml is recommended, since viral evolution and drug resistance mutations may accompany low-level viral replication, subsequently leading to virologic failure (36–39).

However, also the performance characteristics of each individual diagnostic assay should carefully be taken into account when VL values are evaluated and clinical intervention is being considered. For example, when incomplete virologic response is suspected (such as in the presence of repeated low levels of HIV-1 RNA), it is important to consider that the Roche assay produces HIV-1 RNA quantification levels 0.2 to 0.5 log10 higher than those of other real-time-based assays (20, 40–42). For instance, by extrapolating the data from our comparison of clinical samples and HIV-WR1 with HIV-RNA values close to 50 copies/ml, we may foresee that a viral load considered VF as measured with the Roche assay might correspond to a suppressed viral load with the Abbott assay (i.e., 120 versus 40 copies/ml, respectively). It is evident that in such a case, the clinical decision would be different depending on the assay used, and it may benefit from repeated measurement.

LVL represents a pivotal focus in clinical management, as it leads to consequences (43, 44), such as different interpretations of therapy efficacy, possibly leading to relevant changes in therapeutic schemes, and the lack of correlation among the assays may represent a challenge to the interpretation of the results. However, the clinical implications of the present findings are not fully appreciated, and clinical follow-up is necessary to establish whether the differences between the assays are significant enough to make a large difference in treatment decisions.

A large clinical study is necessary to establish whether the GL should take into consideration the performance characteristics of each assay when making statements about HIV-1 RNA cutoffs for clinical decisions.

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