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Comparative performance of the Biocentric Generic Viral Load, Roche CAP/CTM v1.5, Roche CAP/CTM v2.0 and m2000 Abbott assays for quantifying HIV-1 B and non-B strains: Underestimation of some CRF02 strains

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ABSTRACT

Background: HIV-1 viral load testing is now recommended by the World Health Organization for every patient receiving antiretroviral therapy (ART).
Objectives: The objective of this study is to evaluate the performance of commercial assays for their ability to quantify HIV-1 strains currently circulating in France.
Study design: The performances of the Generic HIV-RNA assay from Biocentric were compared to those of the Roche CAP/CTM v1.5, Roche CAP/CTM v2.0 and Abbott m2000 RealTime HIV-1 assays. A total of 1885 HIV-1 plasma samples were tested, including 684 samples from patients included in the ANRS-Primo Cohort.
Results: We found a good concordance of quantification between the Roche v2.0 and the Biocentric assays, both of which were superior to the Roche v1.5 assay. We show moderate agreement between techniques; however, CRF02_AG strains and undetermined viruses were underestimated when quantified with the Roche CAP/CTM v2.0. In contrast, a comparison of the Biocentric and Abbott assay results showed strong agreement between assays, indicating that both are well suited for quantification of CRF02_AG strains. Moreover, a 2% underestimation of the B subtypes was observed with the Biocentric assay.
Conclusions: These results have implications for viral load monitoring in Western Africa, where CRF02_AG strains are highly prevalent. Closer epidemiological surveillance and evaluation of commercial assays are still necessary to better evaluate the impact of the genetic evolution of circulating viruses on HIV-RNA quantification in the regions most affected by the HIV-1 epidemic.

1. Background

The ambitious global 90-90-90 target of UNAIDS for the year 2020 is a substantial challenge [1]. The World Health Organization [2] guidelines recommend HIV viral load (VL) for every patient receiving antiretroviral therapy (ART) once a year [2], initially to detect treatment failure. Currently, the objective of the third target, that 90% of treated patients have a controlled VL, is considered a marker of treatment success. Because more than 18 million patients receive treatment worldwide, the need for VL tests is already important and it will increase in the future, while laboratories' implementation in resource-limited settings is still a major issue [3]. The need for involvement of manufacturers, donors and government authorities is urgent to connect all the links in the chain necessary to ensure that these objectives can be

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achieved [4]. The precision and accuracy of HIV-RNA quantification are critical as VL is the major biological marker of treatment efficacy. But in regions most affected by the epidemic, the HIV-1 genetic diversity is important and is steadily increasing, mainly due to genetic recombination phenomena [5]. Therefore, it is necessary to maintain long-term surveillance of VL assays regarding their ability to accurately quantify the different circulating variants. Several studies have reported discrepancies among previous assays and underestimations of VL levels, especially for HIV-1 non-B subtypes; including HIV-1 subtype C and CRF02_AG [6–9].

Surveillance of the molecular epidemiology of viruses from patients with primary infection has shown an evolution of HIV-1 diversity in France in recent years, with an increase of HIV-1 group M, non-B subtypes, including CRF02_AG [10]. This viral diversity could be partly explained by multiple contacts with people from French-speaking Western and Central African countries.

2. Objectives

The objective of this study is to evaluate the recent performance of commercial assays for quantifying HIV-1 strains currently circulating in France. We evaluated the Generic HIV Viral Load assay (Biocentric, Bandol, France) in comparison with the Roche CAP/CTM v1.5, Roche CAP/CTM v2.0 and Abbott m2000 RealTime HIV-1 assays.

3. Study design

3.1. Sample collection

Four teams from the ANRS–AC11 HIV Quantification Working Group participated in this study. Plasma samples with positive HIV-RNA was selected from the Virology Laboratory in Necker Hospital (1030), including 684 samples obtained at study inclusion from January 2007 to December 2014 from patients included in the multicentric ANRS-Primo Cohort.

Positive HIV-RNA samples (N = 855) monitored routinely in the Virology Laboratories of Caen, Rennes and Rouen that were selected during the same time period.

3.2. HIV-1 RNA quantification

HIV RNA quantification was performed according to the manufacturer's instructions for each assay.

The Biocentric assay is a CE-IVD-labeled quantitative real-time PCR assay targeting the *LTR* gene. The plasma volume tested was 0.2 mL, the limit of quantification (LOQ) was 300 copies/mL (2.48 log). The Roche v1.5 is a real-time PCR assay that targets the *gag* gene, while the upgraded Roche v2.0 targets the *gag* and *LTR* genes; the plasma volume tested was 1 mL, and the LOQ values are 50 copies/mL and 20 copies/mL for the v1.5 and v2 versions, respectively. The Abbott assay amplifies the integrase gene; the input volume was of 0.6 mL, and the LOQ is 40 copies/mL.

3.3. HIV-1 subtypes

The HIV-1 subtypes were determined in each laboratory by sequencing and phylogenetic analyses of the *pol* gene, as previously described [10].

3.4. Statistical analysis

EDTA plasma HIV-RNA values were expressed as the $\log_{10}$ value of the copy number per mL. Comparisons were performed using Spearman rank correlation coefficients and Bland-Altman analysis, to assess agreement between the methods (Prism software). Because a difference of 0.5 $\log_{10}$ value of the copy number per mL is the cutoff usually considered in clinical practice, we choose a higher cut-off of 0.7 $\log_{10}$ between values obtained by two methods as the limit of maximum acceptable differences. For all comparisons, we calculate the percentage of samples with a difference of values higher than 0.7 $\log_{10}$ copies per ml.

4. Results

Among the 1885 HIV-1 plasma samples tested, 1030 were tested in the Virology Laboratory of Necker Hospital; a total of 328 samples were tested with the Roche CAP/CTM v1.5 between 2007 and 2008, while 702 samples were tested with the Roche CAP/CTM v2.0 between 2008 and 2014. The Biocentric assay was performed on all samples in parallel.

Over the same period, 855 plasma samples from the Virology Laboratories in Rouen (N = 579), Rennes (N = 253) and in Caen (N = 23) were quantified with the Abbott m2000 RealTime HIV-1 assay routinely used in those three labs. All samples were then tested with the Biocentric assay.

4.1. Comparison of the Biocentric and Roche v1.5 assays

As presented in Fig. 1A, the Spearman correlation analysis of results obtained from the first panel of 328 plasma samples tested with the Biocentric and Roche v1.5 assays yielded an R2 = 0.759 ($p < 0.0001$). The Bland-Altman analysis showed overall agreement, with a median difference of +0.4 log in favor of the Biocentric assay (Fig. 1B) and a standard deviation (SD) at 0.6. Differences exceeding +0.7 log copies/

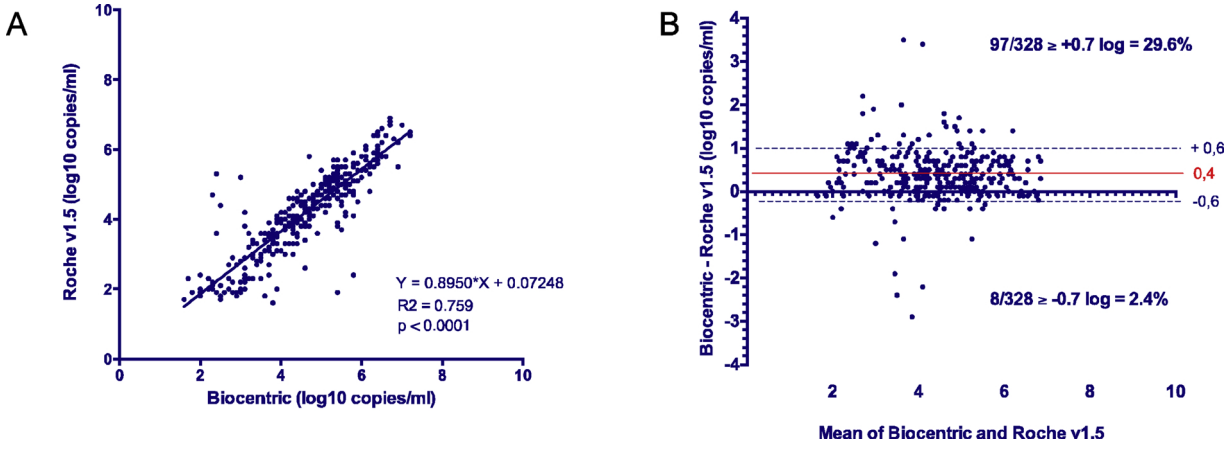

Fig. 1. Comparison between the Biocentric and Roche v1.5 assays: Fig. 1A and B (328 plasma samples).

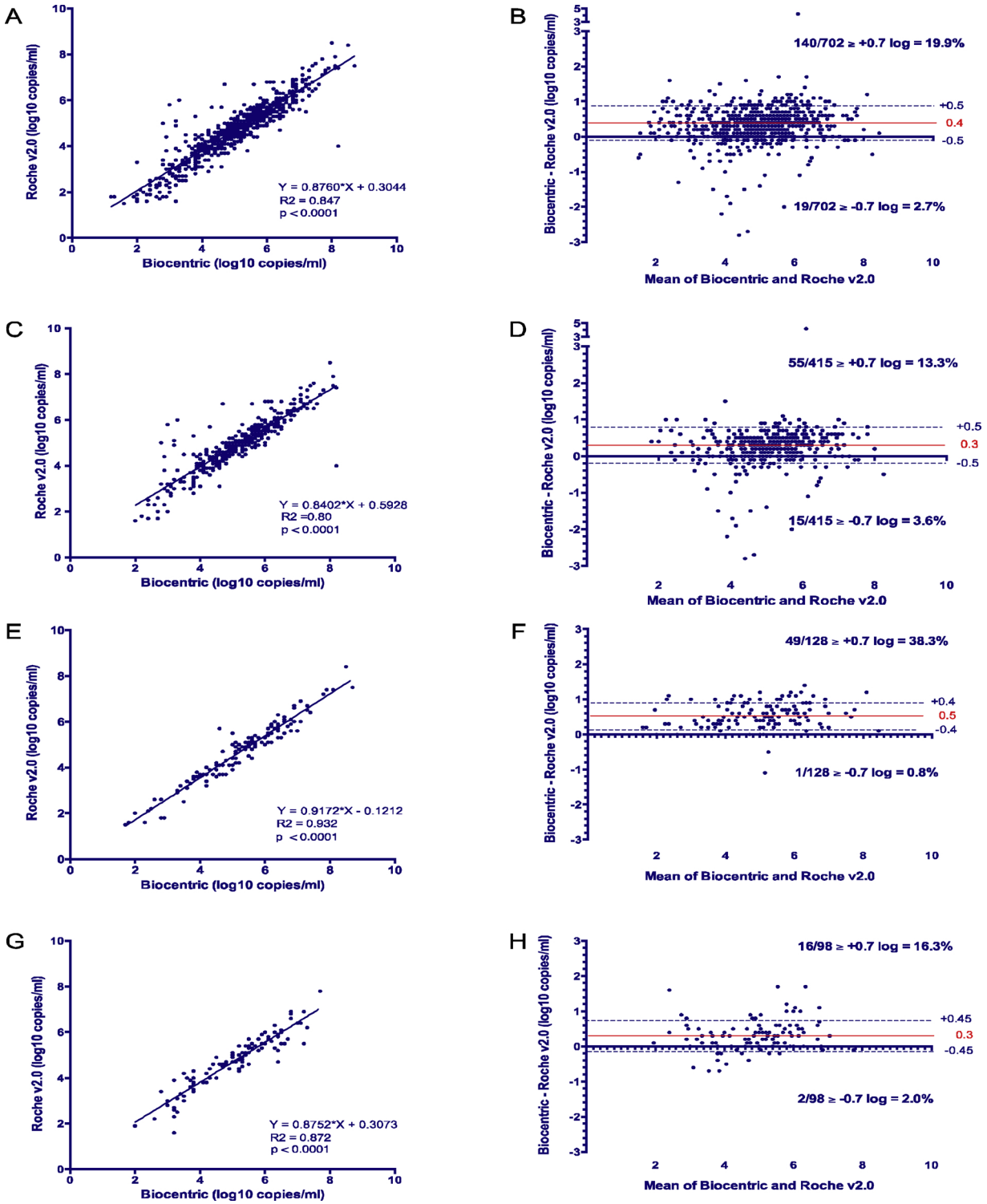

Fig. 2. Comparisons between the Biocentric and Roche v2.0 assays: Fig. 2A and B (all 702 samples), Fig. 2C and D (415 HIV-1 B subtypes), Fig. 2E and F (128 HIV-1 CRF02 subtypes), Fig. 2G and H (98 HIV-1 Non-B and non-CRF02 subtypes).

mL were found in 97/328 samples indicating an underestimation of 29.6% of samples tested with the Roche assay and of 2.4% (8/328) of samples tested with the Biocentric assay.

4.2. Comparison of the Biocentric and Roche v2.0 assays

The Spearman correlation analysis of results obtained for the second panel of 702 plasma samples tested with the Biocentric and Roche v2.0 assays yielded an $R2 = 0.847$ ($p < 0.0001$) (Fig. 2A). The Bland-Altman analysis showed an overall agreement with a median difference of +0.4 log in favor of the Biocentric assay (Fig. 2B) and a SD = 0.5. Differences exceeding +0.7 log copies/mL were found in 140/702 samples, indicating an underestimation in 19.9% of the samples tested with the Roche assay and 2.7% (19/702) of the samples tested with the Biocentric assay.

Among these 702 samples, the HIV-1 subtype was obtained for 648

samples (54 strains were not amplified), which included 415 B, 128 CRF02_AG, 98 non-B and non-CRF02_AG subtypes and 7 samples with an undetermined subtype. The 98 samples with non-B and non-CRF02_AG subtypes included 14 A, 2 B/C, 11 C, 15 CRF01, 3 CRF06, 1 CRF07, 1 CRF09, 1 CRF11, 1 CRF12, 5 CRF14, 1 CRF15, 1CRF22, 1 CRF24, 1 CRF27, 1 CRF 28/29, 2 CRF 45, 1 CRF 47, 7 D, 15 F, 6 G, 5 H, 2 URF and 2 complex strains.

For the 415 HIV-1 subtype B strains, the Spearman correlation analysis (Fig. 2C) yielded an R2 = 0.80 ($p < 0.0001$), and the Bland-Altman analysis indicated that the median of difference was +0.3 log in favor of the Biocentric assay and a SD at 0.5 (Fig. 2D). In 55/415 samples, the difference exceeded +0.7 log copies/mL, indicating that 13.3% of the samples were underestimated with the Roche v2.0 assay, while 15/415 (3.6%) samples were underestimated with the Biocentric assay. While the samples underestimated with Roche v2.0 assay had concentrations lower than 2 log copies/mL, samples underestimated by Biocentric showed a larger difference, with 1% concentrations higher than 2 log copies/mL (n = 4).

For the 128 CRF02_AG strains, the Spearman correlation analysis (Fig. 2E) yielded an R2 = 0.932 ($p < 0.0001$), and in the Bland-Altman analysis, the median difference was +0.5 in favor of the Biocentric assay and a SD at 0.4 (Fig. 2F). In 49/128 samples, the difference exceeded +0.7 log copies/mL, indicating that 38.3% were underestimated with the Roche v2.0 assay, while 1/128 samples (0.8%) were underestimated with the Biocentric assay.

For the 98 HIV-1 non-B/ non-CRF02_AG subtypes, the Spearman correlation analysis yielded an R2 = 0.872 ($p < 0.0001$) (Fig. 2G), and the Bland-Altman analysis showed a median difference of +0.3 in favor of the Biocentric assay and SD at 0.45 (Fig. 2H). In 16/98 samples, the difference exceeded +0.7 log copies/mL, indicating that 16.3% were underestimated with the Roche v2.0 assay, while 2/98 samples (2.0%) were underestimated with the Biocentric assay.

4.3. Comparison of the biocentric and Abbott assays

This part of the study (Fig. 3) included 855 samples with 244 HIV-1 B, 150 CRF02_AG, 114 non-B/non-CRF02_AG (the genotype was not available for 347 samples).

For the 855 samples, the Spearman correlation analysis yielded an R2 = 0.845 ($p < 0.0001$), indicating a strong agreement between the two assays (Fig. 3A). The Bland-Altman analysis showed a median difference of +0.2 in favor of the Biocentric assay and a SD at 0.46 (Fig. 3B). The results were very similar for the three laboratories (data not shown). In 69/855 samples, the difference exceeded +0.7 log copies/mL, indicating that 8.1% were underestimated by the Abbott assay, while 29/855 (3.4%) were underestimated by the Biocentric assay. Altogether, these results indicated strong agreement between the Biocentric and Abbott assays.

For 244 B subtype samples, the Spearman correlation (Fig. 3C) analysis yielded an R2 = 0.76 ($p < 0.0001$). The Bland-Altman analysis (Fig. 3D) showed a median difference of +0.2 log copies/mL in favor of the Biocentric assay and a SD at 0.61. For 25/244 samples, the difference exceeded +0.7 log copies/mL, indicating that 10.2% were underestimated with the Abbott assay, while 15/244 (6.1%) were underestimated with the Biocentric assay, but the differences were higher than 2 log copies/mL in 2% of the samples (n = 5).

For 150 CRF02_AG samples, the Spearman correlation analysis yielded an R2 = 0.928 ($p < 0.0001$) (Fig. 3E), indicating a strong agreement between the two assays. The Bland-Altman analysis showed a median difference of +0.3 in favor of the Biocentric assay, and a SD at 0.33 (Fig. 3F). For 16/150 samples, the difference exceeded +0.7 log copies/mL, indicating that 10.7% were underestimated with the Abbott assay, while 1/150 (0.7%) was underestimated with the Biocentric assay.

The 114 other HIV1 samples included 27 A, 21 C, 3 CRF01, 6 CRF06, 4 CRF11, 3 CRF12, 3 CRF14, 1 CRF15, 1 CRF18, 1 CRF22, 1 CRF31, 1 CRF42, 2 CRF60, 5 D, 13 F, 16 G, 3 H, 2 J, and 1 K subtypes. The Spearman correlation (Fig. 3G) analysis yielded an R2 = 0.915 ($p < 0.0001$). The Bland-Altman analysis (Fig. 3H) showed a median difference of +0.25 in favor of the Biocentric assay, with a SD at 0.4. For 9/114 samples, the difference exceeded +0.7 log copies/mL, indicating that 7.8% were underestimated with the Abbott assay, while 3/114 (2.6%) were underestimated with the Biocentric assay.

5. Discussion

HIV-1 viral load monitoring is critical to ensure and maintain an effective treatment response or detect virological failure. Commercial viral load tests were initially optimized to quantify HIV-1 group M, and selected primers and probes have been adapted to subtype B, which predominates in the US and Europe. The global distribution of HIV-1 subtypes continues to differ by geographical regions with an increase in recombination most notably in CRF strains as noted by the large burden of CRF02_AG in Western Africa. This study focuses on VL quantification of HIV-1 strains circulating in France in recent years; it includes a large number of various non-B and CRF02 subtypes.

The results show relatively good agreement between the Roche v1.5 and the Biocentric assays; the correlation with the Biocentric assay was even better for the Roche v2.0 assay, which is currently the most used version. We chose to classify a high difference of 0.7 log copies/mL as a significant discrepancy to evaluate the percentage of samples underestimated by each assay. Compared to the Biocentric results, the percentage of underestimation decreased from 29.6% to 20% for the Roche v1.5 and v2.0, respectively. We confirmed the marked improvement of the Roche v2.0 assay noted in previous reports [8,11] for viruses of patients recently infected in France.

The median difference of +0.3/+0.4 log in favor of the Biocentric assay compared to both Roche assays raises the question of whether this difference indicates better quantification or an overestimation by the Biocentric assay. It must be noted that the different standards for HIV-1 quantification of the Biocentric assay were tested with the Roche v2.0 assay; the results were completely concordant with the expected values indicated by the manufacturer (data not shown). Therefore, the difference in quantification cannot be related to the method of quantification. A difference of approximately +0.3 log has been already reported in previous studies [12–14].

We report an overall good concordance of quantification between the Roche v2.0 and Biocentric assays, showing that they are adapted to quantify different subtypes. In contrast, our results show that the agreement was moderate but inconsistent for CRF02_AG and undetermined viruses by the Roche v2.0 assay. Conversely, the comparison of results obtained with the Biocentric and the Abbott assays showed strong agreement, suggesting that the Biocentric assay does not overestimate CRF02_AG loads. Therefore, we conclude that the current Roche v2.0 assay underestimates many CRF02_AG strains, while they are major variants predominant in West and Central Africa and have undergone multiple genetic recombination during their evolution [15,16]. A recent publication provides a detailed description of the CRF02_AG diversity, classifying strains into six diverse monophyletic clusters [17].

We observed underestimations with few B subtypes by the Biocentric assay compared to the Roche and Abbott assays. The high HIV-1 diversity can partially explain these variations and underestimations. Recombination may also occur between viral subtypes in dually infected individuals, including within the amplified region of the LTR gene. A poor performance of the Nuclisens (from BioMérieux) assay for plasma samples harboring CRF02_AG strains has been previously reported [7], and a global disparity was also observed with more recent assays for CRF02_AG strains [12].

Accurate measurement of VLis critical and can have an important clinical impact. Underestimation of more than one log may not detect a continuous replication and/or blips that may have important

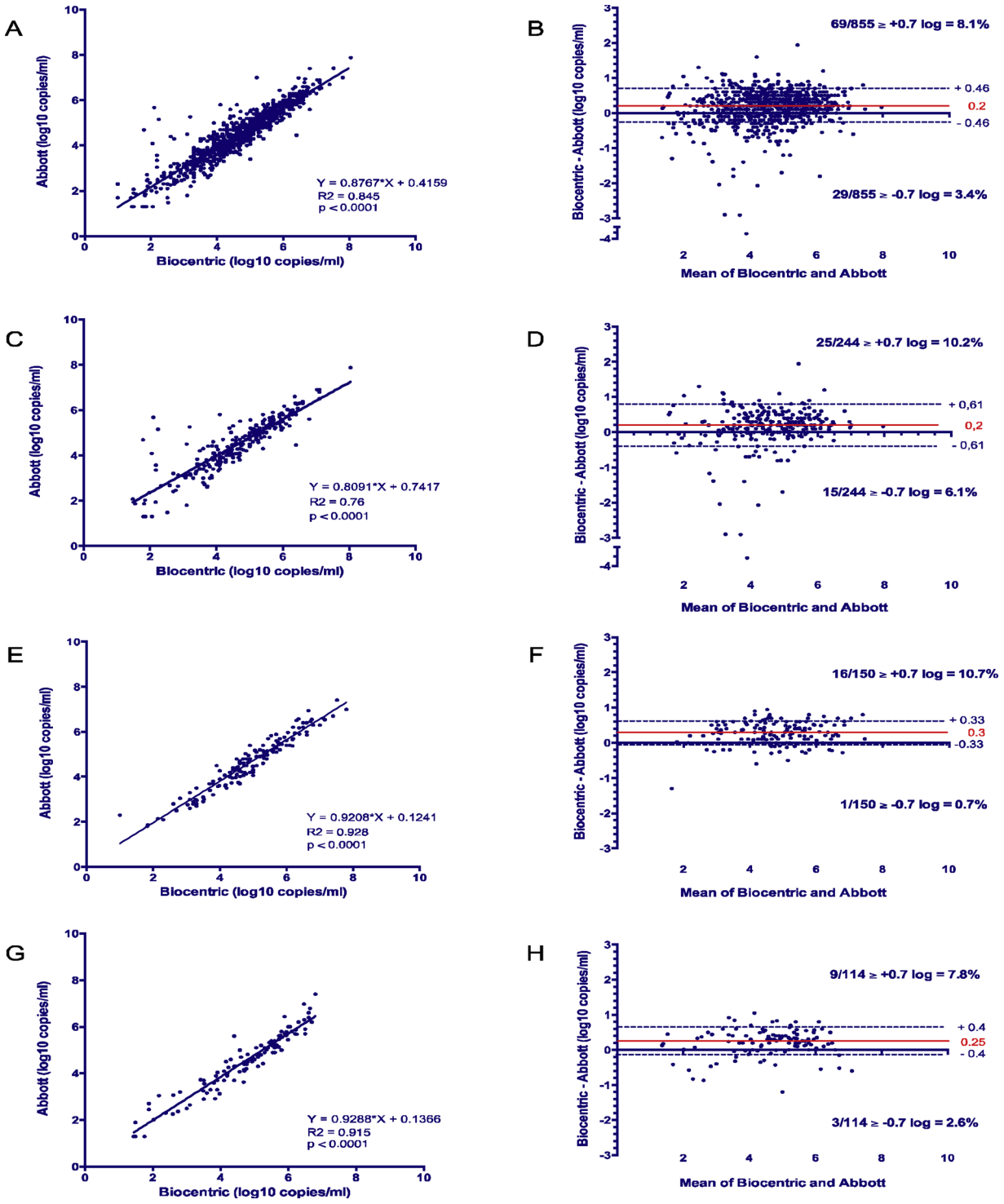

Fig. 3. Comparisons between the Biocentric and Abbott assays: Fig. 3A and B (all 855 samples), Fig. 3C and D (244 HIV-1 B subtypes), Fig. 3E and F (150 HIV-1 CRF02 subtypes), Fig. 2G and H (114 HIV-1 Non-B and non-CRF02 subtypes).

consequences. Some patients may be incorrectly considered efficiently treated, while at risk of developing resistance to ART. When VL is inconsistent with clinical data and/or the CD4 + T cell count, physicians should be aware of possible underestimations. We recently observed two patients receiving ART with VL was undetectable with the Roche 2.0 assay, but > 1000 copies/mL with the Biocentric assay (data not shown). We suggest that patients should be monitored not only by the same method, but also with the best method adapted to their virus. In laboratories receiving samples with high diverse subtype, performing a second technique in parallel may be necessary.

Our results confirm that differences in performance between assays persist [19–22] and that current assays are not equivalent, as reported for the Nuclisens assay not adapted to subtype C [6,24]. It is obvious that no perfect technique exists, and it is challenging to overcome the difficulty of amplifying all HIV subtypes while genetic diversity continues to expand. Our results confirm that the Biocentric assay appears well suited for VL in African countries, where CRF02_AG strains are highly prevalent. Moreover, this assay can be used on open polyvalent

platforms (OPP) that include a simple independent automated nucleic acid extractor and a real-time PCR device [23].

In conclusion, discrepancies still exist despite overall good agreement between assays. Our study reveals the importance of interpreting with caution VL measurements of CRF02_AG strains using the Roche v2.0 assay and of few B subtypes when using the Biocentric assay. This has implications for resource-limited settings; a closer and constant surveillance is necessary to better evaluate the impact of genetic evolution of circulating virus on HIV-RNA quantification [25]. Similar studies should be performed regularly in different geographical areas, particularly in areas with high HIV-1 genetic diversity and especially in Western African countries where new recombinant forms circulate, including CRF02_AG. Such assessments are necessary to select the most practical and suitable assays in each country and to allow decision-makers to optimize assay choices that are well adapted to the needs of resource-limited countries.

Author contribution

VAF, AM, JCP and CR designed the work and drafted the manuscript; AM, MG, AM, DJ, MG, MLC executed the experiments; JPV, ND contributed to the patient care and LM to the plasma collection (ANRS Primo Cohort). All authors revised the manuscript and approved the manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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Appendix A

The ANRS-AC11 HIV Quantification Working Group

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