Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Original Article

Comparative Performance of the Biocentric Generic Viral Load, and M2000 Abbott Assays for Quantifying HIV-1 in Cameroon

Étude comparative des tests Biocentric Generic Viral Load et M2000 Abbott pour la quantification du VIH-1 au Cameroun

Medi Sike CI[1,2*], Mbebi Enone JP[3], Lobe SA [3], Voundi Voundi E[2], Ngogang MP[2,] Ndoumba Mintya A[2], Embolo Enyegue EL[4], Essomba EN[3], Luma Henry[2].

1.Department of laboratory, Douala Laquintinie Hospital, Douala, Cameroon
2.Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon
3.Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon
4.Ministry of Scientific Research and Innovation, Yaounde, Cameroon

Auteur correspondant :
Medi Sike CI
Tel: +237699196274
Email: chritingm@yahoo.fr

Keywords: Humans, RNA, HIV Seropositivity, Molecular Biology, Recombination.

Mots clés Humains, ARN, Séropositivité VIH, Biologie Moléculaire, Recombinaison.

ABSTRACT

Background. Molecular biology quantification of plasma viral Ribonucleic Acid (RNA) is a biological marker of treatment failure or success through Viral Load (VL). The Human immunodeficiency virus type 1 (HIV-1) genetic diversity is important and is steadily increasing, mainly due to genetic recombination phenomena and drug pressure. The aim of this study was to compare two platforms of HIV-RNA quantification targeting two different regions. **Methods.** The performance of the Generic HIV-RNA assay from Biocentric (Bandol – France) was compared to the Abbott m2000 Real-Time HIV-1 (Abbott Molecular Inc. Des Plaines, USA) in Laquintinie Hospital. A total of 84 HIV-1 plasma samples were tested, the Cohen's Kappa concordance, spearman correlation, Bland-Altman analysis, and Lin's concordance were analyzed using Statistical Package for the Social Sciences (SPSS) 20.0 software; the significance was set at 5%. **Results.** The proportion of patients with no detectable viral load was 44.0% (37/84) with Abbott versus 65.5% (55/84) with Biocentric. The Cohen's Kappa coefficient was 0,16 (95% CI: -0,08 to 0,39) showing slight agreement. The results were clinically concordant for 64/84 samples. The Bland Altman analysis revealed a bias of -0.49 (CI=-1.51-0.54) Log10 copies/mL in favor of Biocentric indicating an underestimation of Viral Load (VL) by the Abbott technique. The range of 95% limits of agreement of (-3.50 to 2.51) Log10 copies/mL was wide compared to the clinically acceptable threshold (+/-0.5 Log10 copies/mL). **Conclusion.** The two methods used to quantify viral load in our context are not well correlated and there is a poor agreement, sequencing should be done routinely and reagent adapted.

RÉSUMÉ

Contexte. La quantification par biologie moléculaire de l'acide ribonucléique (ARN) viral plasmatique est un marqueur biologique de l'échec ou du succès du traitement par détermination de la charge virale (CV). La diversité génétique du virus de l'immunodéficience humaine de type 1 (VIH-1) est importante et en augmentation constante, principalement en raison des phénomènes de recombinaison génétique et de la pression médicamenteuse. Le but de cette étude était de comparer deux plates-formes de quantification de l'ARN-VIH ciblant deux régions différentes. **Méthodes.** Les performances du test Generic HIV-RNA de Biocentric (Bandol – France) ont été comparées à celles du test Abbott m2000 Real Time HIV-1 (Abbott Molecular Inc. Des Plaines, USA) à l'hôpital Laquintinie. Au total, 84 échantillons de plasma VIH-1 ont été testés, la concordance Kappa de Cohen, la corrélation de Spearman, l'analyse de Bland-Altman et la concordance de Lin ont été analysées à l'aide du logiciel Statistical Package for the Social Sciences (SPSS) 20.0 ; la significativité a été fixée à 5 %. **Résultats.** La proportion de patients sans charge virale détectable était de 44,0 % (37/84) avec Abbott versus 65,5 % (55/84) avec Biocentric. Le coefficient Kappa de Cohen était de 0,16 (IC à 95 % : -0,08 à 0,39) montrant une très légère concordance. Les résultats étaient cliniquement concordants pour 64/84 échantillons. L'analyse de Bland Altman a révélé un biais de -0,49 (IC=-1,51-0,54) Log10 copies/mL en faveur de Biocentric indiquant une sous-estimation de la charge virale (CV) par la technique d'Abbott. La plage des limites d'accord à 95 % de (-3,50 à 2,51) Log10 copies/mL était large par rapport au seuil cliniquement acceptable (+/-0,5 Log10 copies/mL). **Conclusion.** Les deux méthodes utilisées pour quantifier la charge virale dans notre contexte ne sont pas bien corrélées et il y a un mauvais accord, le séquençage doit être fait en routine et les réactifs adaptés.

HIGHLIGHTS

What is already known on this topic

Despite the high diversity of HIV in Cameroon, open techniques for HIV viral load follow-up such as Biocentric with a good concordance with Abbott techniques.could be useful.

What question this study addressed

Correlation between Biocentric and Abbott techniques after the transition to dolutegravir -based regimens for first-, second- and/or third-line anti-retroviral treatment

What this study adds to our knowledge

Due to HIV mutations, the methods used to quantify viral load in our context may not be well correlated

How this is relevant to practice, policy or further research

Special attention should be addressed to continuous surveillance and reagent should be adapted to circulating strains.

INTRODUCTION

The HIV pandemic is a major public health issue worldwide, particularly in Africa and Cameroon (1). Globally, 38.4 million (33.9 million–43.8 million) people were living with HIV in 2021. Of all people living with HIV, 85% (75–97%) knew their status, 75% (66–85%) were accessing treatment and 68% (60–78%) were virally suppressed in 2021. Among people who knew their status, 88% (78– >98%) were accessing treatment and 92% (81–>98%) of them were virally suppressed (2). West and Central Africa is the third most affected region in the world with 3.9 million people living with HIV (2). The global 95-95-95 target of the United Nations Programme on HIV/ acquired immunodeficiency syndrome (AIDS) (UNAIDS) for the year 2025 is a challenge since the current UNAIDS strategy was concluded in 2021 (3). HIV replication is the major pathophysiological event during this affection, this is a biological maker of treatment failure or success through VL. The World Health Organization (WHO) guidelines recommend HIV VL for every patient receiving antiretroviral therapy (ART) once a year and 95% of treated patients should have a controlled VL(4). Molecular biology quantification of plasma viral RNA has emerged as the gold standard test for assessing the level of viral replication. At the end of 2021, 21.4 billion dollars (in constant 2019 United States dollars) was available for the AIDS response in low- and middle-income countries; around 60% was from domestic sources (2). 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 (5). VL quantification is still not widely available in Africa, mainly because of its cost and the lack of laboratory equipment, especially in rural areas. Cameroon has benefited from the support of various non-governmental organizations **(**NGOs) that have enabled the acquisition of different VL measurement platforms, namely the Biocentric open platform and Abbott m2000. The supply of VL should not be interrupted and in case of unavailability of one of the platforms, particularly due to stockout of reagents or equipment maintenance parts, common in the country, these platforms should be interchangeable to guarantee the continuity of virological monitoring of people living with HIV (PLHIV).On the other hand, in regions most affected by the epidemic, the HIV genetic diversity is important and is steadily increasing, mainly due to genetic recombination phenomena (6) and drug pressure. It is necessary to maintain 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 Circulating Recombinant Form 02_AG (CRF02_AG) (7) (8) (9) (10). Surveillance of the molecular epidemiology of viruses from patients with infection has shown an evolution of HIV-1 diversity in Cameroon including CRF02_AG as the major recombinant form (11). The current transition to a Dolutegravir (DTG)-based ART is predicted to be very effective in rapidly controlling viral replication (12) (13). This viral diversity could be partly explained by multiple recombination and mutation due to drug pressure even for new treatment as reported for integrase inhibitors in Cameroon (14) (15). The aim of this study was to compare two platforms of HIV-RNA quantification targeting two different regions in a plasma of PLHIV.

METHODS

Study design and setting

A prospective study was carried out over a period of 2 months, in Douala Laquintinie Hospital, 2 aliquots of 2ml of Plasma sample were received and stored at -80°C till the date of analysis by the Abbott m2000 and Biocentric Platform in parallel in the Virology Laboratory of Laquintinie Hospital. Routinely, in our laboratory, the VL is measured either on Biocentric or Abbott m2000 depending on the availability of the techniques.

Inclusion and exclusion criteria

Samples that did not have VL results on one or both platforms were excluded from the study.

Sampling Procedure

The sample size was calculated based on the formula for basic sample size calculation for random sampling (15). The 95% confidence level and 3.7% prevalence of HIV in Cameroon were used (16) and 54 samples were required for the minimum sample size. A consecutive and non-probabilist method was used. After measuring the VL of 100 samples received, 16 samples did not have results on one or both platforms, forming an exclusion rate of 16% the total number of samples included in the study was 84.

Sample assessment

Specimen collected in plasma Ethylène Diamine tetracetic acid (EDTA) was used, Freshly drawn specimens (whole blood) were held at 15-30°C for up to 6 hours or at 2-8°C for up to 24 hours, prior to centrifugation. After centrifugation at 2000g/minute for 15 minutes, plasma was removed from cells and stored at -70°C or lower till the date of analysis. Multiple freeze thaw cycles were avoided and did not exceed three freeze/thaw cycles.

Plasma specimens were Thawed at 15-30°C or 2-8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2-8°C for up to 6 hours.

HIV-1 RNA quantification

This was performed according to the manufacturer's instructions for each assay.

The Biocentric assay is a quantitative real-time PCR assay targeting the 3'LTR gene among HIV-1 group M subtypes (17). The plasma volume tested was 0.2 mL, the Limit Of Detection (LOD) is 388 copies/mL (2.48 log) and the specificity is 100%. The Generic HIV-RNA assay from Biocentric (Bandol – France) test does not allow quantifying the RNA of HIV-1 group O.

The internal control (IC) is added directly to each sample during the extraction step, and consists of an RNA sequence unrelated to the HIV-1 target sequence; The RNA extraction and then the purification procedure uses the principle of magnetic beads to capture RNA.

Viral RNA extraction was done with GXT NA Extraction Kit (Hain Lifescience GmbH, Nehren, Germany) in the Arrow or GenoXtract extractor (Nordiag, Diasorin, Dublin, Ireland).

The amplification was carried out with the QuantStudio™ 7flex thermocycler (Thermo fisher scientific, applied biosystems, Singapore), the set of oligonucleotide primers and the IC probe are labeled with the fluorochrome reporter Cy5™, this was added during the preparation of the mixture of Polymerase Chain Reaction (PCR).

The method uses the fluorochrome-labeled probes ROX™ and 5' FAM™ as reporters. The standard curve is validated according to the manufacturer's criteria

Interpretation of the results of the Generic HIV-RNA assay from Biocentric (Bandol – France) test is :

- The Internal Control had to be valid for both the extraction and the amplification procedure. The Cy5 Cycle Threshold value should be between 23 and 36. In case the IC RNA of a non-detected sample is not amplified or shows a Ct value ≥40, the patient sample must be re-extracted and re-tested
- HIV-1 viral load values are considered positive for quantification ≥ 388 copies/mL (2.58 log10 copies/mL).
- Results with a viral load detected below this threshold can be considered positive, but the viral load value is less precise and noted as "positive and detected below the test sensitivity threshold".
- A result is declared as "undetectable" when no Ct value for HIV-1 (FAM™ fluorophore) is obtained.
- Unknown samples with Ct values in a non-linear range (below 388 copies/mL or above 5.106 copies/mL) cannot be quantified and must be diluted.

The Abbott RealTime HIV-1 assay was done using the Abbott m2000sp (Abbott Molecular Inc, Thouty Ave, USA) and Abbott m2000rt (Abbott Laboratories, Singapore); the method uses PCR technology with homogenous real-time fluorescent detection. Partially double-stranded fluorescent probe design allows detection of diverse group M subtypes, group O and N isolates (18). The Abbott assay amplifies the integrase gene; the input volume was 0.6 mL, the LOD is 40 copies/mL and the limit of linearity (LL) is 10.000.000 copies/mL. The specificity is 100% (17). All subtypes and groups from Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O, and Group N are detected

The HIV-1 unrelated RNA sequence target sequence is introduced into each specimen at the beginning of sample preparation and simultaneously amplified by RT-PCR, and serves as an IC.

The extract is done on the Abbott m2000sp™ (Abbott Molecular Inc, Thouty Ave, USA) using the Abbott m™ Sample Preparation System (Abbott Molecular Inc, Des Plaines, USA) reagents using the magnetic particle technology.

The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent labeled oligonucleotide probes on the Abbott m2000rt™ (Abbott Laboratories, Singapore) instrument. On the Abbott m2000rt (Abbott Laboratories, Singapore), the target sequence for the Abbott RealTime HIV-1 assay is in the highly conserved pol integrase region of the HIV-1 genome is amplified. RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, Cucurbita pepo.

The concentration of HIV-1 RNA in specimens and controls is calculated from the stored calibration curve, and the results are automatically reported on the m2000rt workstation.

Assay results can be reported in Copies/mL, Log [Copies/mL], International Units (IU)/mL, or Log [IU/mL]; (1 IU = 0.58 copies, 1 copy = 1.74 IU).

The median amplification cycle at which the IC target sequence fluorescent signal is detected in calibration samples establishes an IC Ct validity range

- A result of "Not Detected" signifies that no target was detected.
- A result of "< 1.60 " indicates that target was detected but is less than LOD
- A result of "1.60 to 7.00 Log [copies/mL]" indicates that the target was detected and the concentration falls between 1.6 log copies per mL (LOD) and 7.0 log copies per mL (LL).
- A result of ">7.00 Log [copies/mL]" indicates that the target was detected and is greater than LL, and was diluted.

Ethical consideration

This study met the ethical conditions of research of Helsinki declaration.

Statistical analysis

The Antiretroviral protocols were collected and presented as frequency and percentage. EDTA plasma HIV-RNA values were expressed as copies/ml for qualitative comparisons and named viral **suppression** (not detectable VL, < level of detection, detectable ≥ level of detection and <1000), and **treatment failure** or ≥1000. Cohen's Kappa coefficient was assessed with 95% CI to evaluate

the clinical concordance between the two methods. This clinical concordance was analyzed regarding antiretroviral therapy.

Samples that were classified as detectable ≥ level of detection and <1000 or ≥1000 with both techniques were compared and expressed as the log10 value of the copy number per mL. They were compared using Spearman rank correlation coefficients and Bland-Altman analysis, to assess agreement between the methods (SPSS) and Lin's correlation coefficient of concordance (CCC) (pc) with his 95% Confidence interval (CI) to evaluate the strength of the agreement between 2 measurements of the same variable (Mc bride 2005). The applicability of the BA was verified by the Shapiro-Wilk test. Because a difference of 0.5 log10 value of the copy number per mL is the cut-off usually considered in clinical practice, we choose a higher cut-off of 0.7 log10 between values obtained by the 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 log10 copies per ml. The level of significance was 5%.

RESULTS

Among the 84 samples analyzed, we had 69 (82.1%) patients in first line treatment with Dolutegravir (Table1). The samples were tested in parallel with the two platforms, 11.9% (10/84) had a treatment failure with Abbott and 21.4% (18/84) had a treatment failure with Biocentric. The proportion of patients with no detectable viral load was 44.0% (37/84) with Abbott versus 65.5% (55/84) with Biocentric. The Cohen's Kappa coefficient of clinical agreement was 0,16 (95% CI : -0,08 to 0,39) showing slight agreement.

Distribution of VL according to the two platforms

Among the 84 HIV plasma samples tested in parallel, 20 were quantified with the Abbott m2000 with 13 positives under the LOD and 51 not detectable. Regarding the Biocentric method, 13 were quantified, 1 was positive under the LOD and 37 were not detectable.

Table 1: Frequency of therapeutic protocols in our study population

Protocols	Frequency	Percentage
TDF/3TC/DTG	64	76.2
TDF/3TC/EFV	4	4.8
ABC/3TC/DTG	5	6.0
ABC/3TC/LPVr	1	1.2
TDF/3TC/ATVr	10	11.9
Total	84	100

TDF : Tenofovir ; 3TC : Lamivudine ; EFV : efavirenz ; ABC : Abacavir ; DTG : Dolutegravir ; LPVr : Lopinavir/ritonavir ; ATVr : Atazanavir/ritonavir

The number of samples with a VL >1000 was 18 using the Biocentric platform and 10 using the Abbott platform. We had 5 samples not detected with Biocentric and >1000 with Abbott platform, wether 9 samples with VL >1000 with Biocentric had a not detectable VL with Abbott.

Only 11 samples were both quantified with Abbott and Biocentric platform with a VL ≥ LOD. The results were clinically concordant for 64 (76.2%) samples. the treatment failure was diagnosed with both platforms in 4 cases and the viral suppression was diagnosed with both platforms in 60 cases The result of the two platforms are presented in Table 2

The table 3 below presents the clinical concordance according to the antiretroviral protocol. Among the patients on Dolutegravir, 10/69 had a diagnosis of viral suppression with Abbott versus a diagnosis of treatment failure with Biocentric while 5/69 had viral suppression with Biocentric and treatment failure with Abbott.

Comparison of the Biocentric and Abbott assays

Among the 11 samples that had detectable VL on both platforms, the normality of the distribution of differences was verified, accepted (p = 0.910) and presented in Figure 1.

Figure 2 presents the Spearman correlation analysis of results obtained by testing with the Biocentric and Abbott R2 = 0.39 (p =0.235), this analysis showed a moderate non significant correlation between the two methods.

Table 2 : Distribution of VL according to the two platforms

			VL Biocentric (copies/ml) [N=84]			
			Treatment failure or ≥1000 n(%)=18 (21.4)	detectable ≥level of detection and <1000 n(%)=9 (13.6)	Viral suppression N (%)=66 (78.6) <level of detection n(%)=2 (3.0)	Not detectable n(%)=55 (83.3)
VL Abbott m2000 (copies/ml) N=84	Treatment failure or ≥1000 N(%)=10 (12.0)		4	1	-	5
	Viral suppression N (%)=74 (88.0)	detectable ≥ level of detection and <1000 n (%)=10 (13.5)	4	2	-	4
		<level of detection n (%)=13 (17.6)	1	2	1	9
		Not detectable n (%)=51 (69.0)	9	4	1	37

VL= Viral Load - Level of detection of Abbott=40 copies/ml - Level of detection of Biocentric=388 copies/ml

Table 3 : Distribution of VL according to the two platforms and antiretroviral protocol

	Abbott	Biocentric	
Antiretroviral protocol		treatment failure n=18	Viral suppression n=66
ABC/3TC/DTG n=5	treatment failure	-	1
	viral suppression	-	4
ABC/3TC/LPVr n=1	viral suppression	1	-
TDF/3TC/ATVr n=10	treatment failure	1	1
	viral suppression	2	6
TDF/3TC/EFV n=4	viral suppression	1	3
TDF/3TC/DTG n=64	treatment failure	3	4
	viral suppression	10	47

TDF : Tenofovir ; 3TC : Lamivudine ; EFV : efavirenz ; ABC : Abacavir ; DTG : Dolutegravir ; LPVr : Lopinavir/ritonavir ; ATVr : Atazanavir/ritonavir

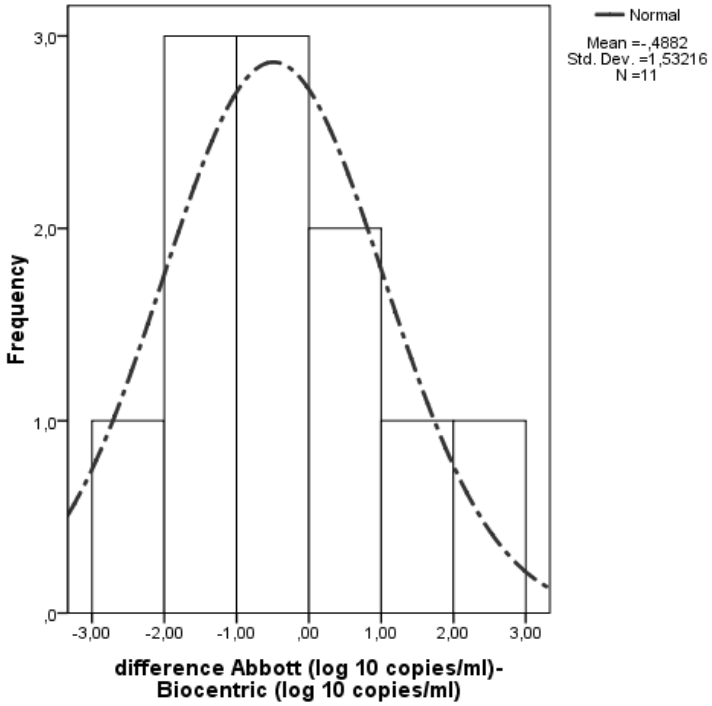


Figure 1 : Distribution of the differences between Abbott and Biocentric platforms

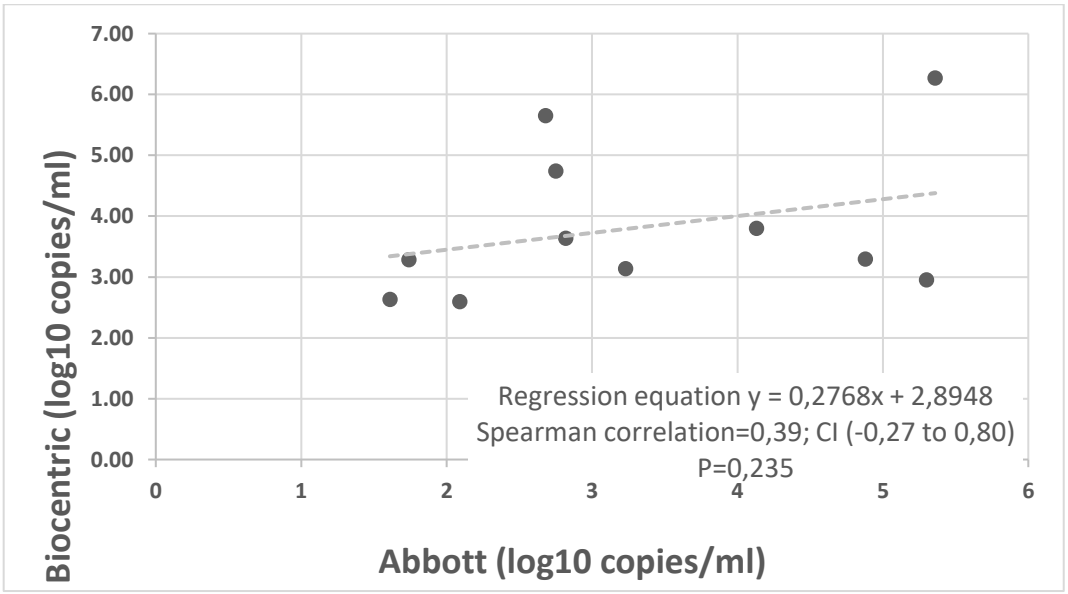
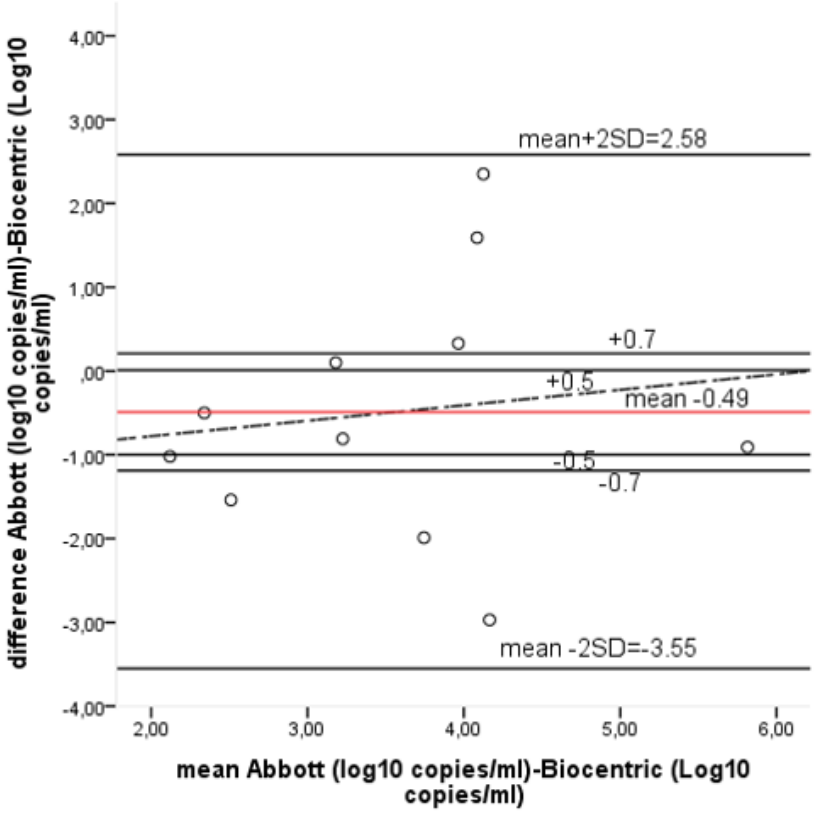


Figure 2 : correlation between the Biocentric and Abbott assays



Regression equation : y = -1,1484 + 0,1849 x

Figure 3 : Bland Altman analysis between the Biocentric and Abbott assays

The Bland Altman analysis is shown in Figure 3, analysis revealed a bias of -0.49 (CI=-1.51-0.54) Log10 copies/mL in favor of Biocentric indicating an underestimation of VL by the Abbott technique. The 95% (lower limit : -3.50 ; (CI=-5,30 to -1,67) upper limit : 2,51 ; (CI=0,69 to 4,33)) Log10 copies/mL range of limits of agreement was wide compared to the clinically acceptable difference (± 0.5 Log10 copies/mL) and the maximum acceptable difference (± 0.7 Log10 copies/mL). Regarding the clinically acceptable difference of 0.5 log 10 copies/ml, 7/11 (63.6%) samples were above the limit, and 6/11 (54.5%) samples were above the 0.7 log 10 copies/ml.

Lin's correlation coefficient of agreement was poor ρ=0.28 [95% CI =-0.29 to 0.71], according to the criteria proposed by Mc Bride (18)

DISCUSSION

This study compared the Abbott and Biocentric HIV-1 viral load Test to assess the equivalence of their results in a resource-limited country with the 95-95-95 goals to be achieved. A total of 84 samples were analyzed in parallel with the two platforms. The proportion of patients with suppressed viral load (11.9% versus 21.4%) on Abbott and Biocentric was not similar ; we evaluated the agreement between the 2 platforms at the threshold of viral suppression (1000 copies/mL) and the Kappa coefficient obtained was 0.16 indicating a slight level of agreement. A discrepancy between 2 analytical methods in clinical biology is acceptable if it does not alter the diagnosis. The concordance was clinically significant for 76.2% of all VL, meaning that for those patients, the treatment management did not changed.

The Correlation coefficient of detected VL with both techniques shows a value of 0.39, this is different from the strong agreement of 0.93 between the two assays obtained by Fatoumata Traoré in Mali and Burkina among 155 samples in 2016 (19), the 0.89 obtained by Ngo Malabo et al in Cameroon in 2017 in 40 samples (20) and the 0.845 obtained by Avettand-Fénoël et al (21) ; The global distribution of HIV-1 subtypes, mutation and CRF continues to differ by geographical regions and time and can explain the discrepancies ; mutations of integrase gene after the introduction of anti integrase in Cameroon should be monitored in our region, after the 2018 WHO recommendation on Dolutegravir introduction as the first line regimen ; especially since the first case of Dolutegravir and Darunavir/r multi drug-resistant HIV-1 in Cameroon was reported by Fokam et al in 2020 (22).

The Bland Altman analysis revealed a difference of 0.48 Log10 copies/mL in favor of VL measured on Biocentric. Abbott seems to underestimate the VL as Avettand-Fénoël et al found ; but, our result was greater than the 0.2 obtained by Avettand et al (21). The range of 95% limits of agreement of (-3.50 to 2.51) Log10 copies/mL was wide compared to the clinically acceptable difference (+/- 0.5 Log10 copies/mL). The level of agreement of the Lin ρ concordance correlation coefficient was poor (0.28) and the 2 platforms were not in satisfactory agreement. This result could be explained by the difference of the 2 platforms in the ability to quantify the different strains of

HI V-1. Indeed, Avettand-Fénoël et al in 2019, showed that the Abbott platform underestimate the CRF02_AG strain With an overall disparity higher for CRF02_AG than for B strains, CRF02_AG is the preponderant HIV-1 strain (> 80%) in Cameroon (21) (23).The genetic diversity of HIV-1 subtypes may present a challenge in monitoring PLHIV with non-B subtypes, thus HIV-1 diversity is increasing in Cameroon (20) (23); drug resistance is also increasing, probably due to factors like drug adherence, pharmacy stock-outs, lack of proper patient retention in care and the use of ARV drugs to prevent mother to child transmission or as pre and post-exposure prophylaxis(24) (25). The number of samples with the maximum clinically significant difference of +-0.7 Log copies/mL (difference between the 2 VL measurements absolute value) was 7/11 (63.6%) ; lower values of 8.1% (underestimation by the Abbott assay) and 3.4% (underestimation by the Biocentric) were reported by Avettand-Fénoël et al; these differences also influence the diagnosis of virological failure (21).

Limitations of the Study

During our study, we faced many difficulties which are as follows: the size of our sample, the absence of HIV RNA sequencing and clinical data; but our findings might be interesting for the management of patients in a developing country to really implement the 95-95-95 goals.

CONCLUSION

The two methods used to quantify viral load in our context are not well correlated and there is a poor agreement, probably due to the virus mutation and or virus recombination, sequencing should be done routinely and reagent should be adapted to the circulating strains.

Acknowledgments

The authors thank the Douala Laquintinie Hospital staff for their kind assistance and advice.

Authors' contributions

MSCI, MEJ, LSA, initiated the project. MSCI, MEJP, LSA conducted biological tests. MSCI, MEJP, LSA, VVE conducted statistical analyzes. MSCI, MEJP, LSA, VVE, NMA, NMP, EEEL, EEN wrote and corrected the manuscript. LH supervised the study. All the authors read and approved the final manuscript.

Competing interests

The authors report no conflict of interest in this work.

REFERENCES

1.Yekayo KD, Fatoumata KK, Cyrielle AT, De Paul YV, Aquin TTD, Hugues A, et al. Comparison of 2 HIV1 RNA VL measurement commercial kits in Cote d'Ivoire. Issues Biol Sci Pharm Res. 11 oct 2021 [cited jan 14th 2023];9(3). Available on: https://journalissues.org/ibspr/wp-content/uploads/sites/6/2021/10/Yekayo-et-al-.pdf

2.UNAIDS_FactSheet_en.pdf. [cited jan 14th 2023]. Available on:https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf

3.2025-AIDS-Targets_en.pdf. [cited jan 14th 2023]. Available on: https://www.unaids.org/sites/default/files/2025-AIDS-Targets_en.pdf

4.World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva: World Health Organization; 2015 [cited jan 14th 2023]. 76 p. Available on: https://apps.who.int/iris/handle/10665/186275

5.Kilmarx PH, Simbi R. Progress and Challenges in Scaling Up Laboratory Monitoring of HIV Treatment. PLOS Med. 23 août 2016;13(8):e1002089.

6.Luft LM, Gill MJ, Church DL. HIV-1 viral diversity and its implications for viral load testing: review of current platforms. Int J Infect Dis. 1 oct 2011;15(10):e661-70.

7.Muenchhoff M, Madurai S, Hempenstall AJ, Adland E, Carlqvist A, Moonsamy A, et al. Evaluation of the NucliSens EasyQ v2.0 Assay in Comparison with the Roche Amplicor v1.5 and the Roche CAP/CTM HIV-1 Test v2.0 in Quantification of C-Clade HIV-1 in Plasma. PLOS ONE. 26 août 2014;9(8):e103983.

8.Holguín A, López M, Molinero M, Soriano V. Performance of Three Commercial Viral Load Assays, Versant Human Immunodeficiency Virus Type 1 (HIV-1) RNA bDNA v3.0, Cobas AmpliPrep/Cobas TaqMan HIV-1, and NucliSens HIV-1 EasyQ v1.2, Testing HIV-1 Non-B Subtypes and Recombinant Variants. J Clin Microbiol. sept 2008;46(9):2918-23.

9.Damond F, Avettand-Fenoel V, Collin G, Roquebert B, Plantier JC, Ganon A, et al. Evaluation of an upgraded version of the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test for HIV-1 load quantification. J Clin Microbiol. avr 2010;48(4):1413-6.

10. No evidence of a change in HIV-1 virulence since 1996 in France. - Abstract - Europe PMC. [cited jan 14th 2023]. Available on: https://europepmc.org/article/med/19424055

11. Semengue ENJ, Armenia D, Inzaule S, Santoro MM, Dambaya B, Takou D, et al. Baseline integrase drug resistance mutations and conserved regions across HIV-1 clades in Cameroon: implications for transition to dolutegravir in resource-limited settings. J Antimicrob Chemother. 13 avr 2021;76(5):1277-85.

12. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV. [cited jan 14th 2023]. Available on: https://www.who.int/publications-detail-redirect/WHO-CDS-HIV-18.51

13. What's New in the Guidelines | NIH. [cited jan 14th 2023]. Available on: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new-guidelines

14. Fokam J, Takou D, Njume D, Pabo W, Santoro MM, Njom Nlend AE, et al. Alarming rates of virological failure and HIV-1 drug resistance amongst adolescents living with perinatal HIV in both urban and rural settings: evidence from the EDCTP READY-study in Cameroon. HIV Med. août 2021;22(7):567-80.

15. Daniel-1995-Biostatistics.pdf. [cited jan 15th 2023]. Available on: https://www.ege.fcen.uba.ar/wp-content/uploads/2014/05/Daniel-1995-Biostatistics.pdf

16. 53059-CAMPHIA-Report_EN_Web_V4.pdf. [cited jan14th 2023]. Available on: https://phia.icap.columbia.edu/wp-content/uploads/2021/04/53059-CAMPHIA-Report_EN_Web_V4.pdf

17. GENERIC HIV Charge Virale. biocentric. [cited jan 21th 2023]. Available on: https://www.biocentric.com/generic-hiv-charge-virale

18. RealTime HIV-1 Viral Load Assay CE | Abbott Molecular. [cited jan 21 2023]. Available on: https://www.molecular.abbott/int/en/products/infectious-disease/realtime-hiv-1-viral-load

19. Torimiro JN, Mafopa NG, Ndongo A, Moyo ST, Lobe EE, Sosso SM, et al. Evaluation of Virologic Methods for Early Detection of HIV-1 in a Resource-limited Setting: Performance

and Cost Analysis. Health Sci Dis. 30 août 2013;14(3). Available on: https://hsd-fmsb.org/index.php/hsd/article/view/222
20. McBride2005.pdf. [cited jan 15th 2023]. Available on: https://www.medcalc.org/download/pdf/McBride2005.pdf
21. Comparaison des Techniques de Mesure de la Charge Virale du VIH-1: Comparaison des techniques de mesure de la charge virale du VIH-1 en Afrique de ... VIH-1 en Afrique de L'Ouest (Mali- Burkina) | Amazon.com.br. Available on: https://www.amazon.com.br/Comparaison-Techniques-Mesure-Charge-Virale/dp/3639503031
22. Ngo-Malabo ET, Ngoupo T. PA, Zekeng M, Ngono V, Ngono L, Sadeuh-Mba SA, et al. A cheap and open HIV viral load technique applicable in routine analysis in a resource limited setting with a wide HIV genetic diversity. Virol J. 14 nov 2017;14(1):224.
23. Avettand-Fénoël V, Mélard A, Gueudin M, Maillard A, Dina J, Gousset M, et al. 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. J Clin Virol. 1 janv 2019;110:36-41.
24. Fokam J, Takou D, Semengue ENJ, Teto G, Beloumou G, Dambaya B, et al. First case of Dolutegravir and Darunavir/r multi drug-resistant HIV-1 in Cameroon following exposure to Raltegravir: lessons and implications in the era of transition to Dolutegravir-based regimens. Antimicrob Resist Infect Control. 26 août 2020;9(1):143.
25. Abongwa LE, Nyamache AK, Torimiro JN, Okemo P, Charles F. Human immunodeficiency virus type 1 ((HIV-1) subtypes in the northwest region, Cameroon. Virol J. 15 août 2019;16:103.
26. Billong SC, Fokam J, Aghokeng AF, Milenge P, Kembou E, Abessouguie I, et al. Population-Based Monitoring of Emerging HIV-1 Drug Resistance on Antiretroviral Therapy and Associated Factors in a Sentinel Site in Cameroon: Low Levels of Resistance but Poor Programmatic Performance. PLoS ONE. 26 août 2013;8(8):e72680.
27. Fokam J, Elat JBN, Billong SC, Kembou E, Nkwescheu AS, Obam NM, et al. Monitoring HIV Drug Resistance Early Warning Indicators in Cameroon: A Study Following the Revised World Health Organization Recommendations. PLoS ONE. 17 juin 2015;10(6):e0129210.