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RESEARCH

Comparison of NucliSens® EasyQ® HIV-1 v2.0 and COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 commercial systems for the determination of viral load of different HIV-1 genetic variants in Cuban patients

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ABSTRACT

The quantification of viral load of the human immunodeficiency virus type 1 in plasma has become an essential tool to the monitoring of infected patients. The aim of this study was to compare the viral load assays, NucliSENS® EasyQ® HIV-1 v2.0 and COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1, in different HIV-1 genetic forms. We collected 109 plasmas from HIV-1 infected patients from Hermanos Ameijeiras Hospital (September 2015 to April 2016). The viral load was quantified using both methods. The viruses were subtyped by in-house sequence-PCR. The medians of viral loads from 54 samples quantified by both methods were different ($p < 0.0001$). The 52 % of the samples had differences higher than 0.5 log IU/mL. Lin's coefficient was 0.86 and Bland Altman's method showed poor concordance between both methods. Viral load levels were different for BGs recombinants between the methods assayed ($p < 0.0091$), being lower with the EasyQ® system. The EasyQ® method sub-quantifies the Cuban CRF_BGs recombinations, which limits the use of this assay for the clinical monitoring of patients infected with this viral variant. We consider that the monitoring of HIV-1 viral load should be performed only by one method for each patient.

Keywords: Viral load, HIV-1 subtypes, NucliSens® EasyQ® HIV-1, COBAS® TaqMan® 48 HIV-1

RESUMEN

Comparación de los sistemas comerciales NucliSens® EasyQ® HIV-1 v2.0 y COBAS AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 para la determinación de la carga viral de diferentes variantes genéticas del VIH-1 en pacientes cubanos. La cuantificación de la carga viral del virus de inmunodeficiencia humana tipo 1 en plasma se ha convertido en una herramienta esencial en el seguimiento de los pacientes infectados. El objetivo de este estudio fue comparar los ensayos de carga viral, NucliSENS® EasyQ® HIV-1 v2.0 y COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1, en las diferentes formas genéticas virales que circulan en Cuba. Se estudiaron 109 plasmas de pacientes infectados que asistieron a la consulta del Hospital Hermanos Ameijeiras (septiembre/2015 a abril/2016). La carga viral se cuantificó por ambos métodos, los virus se subtiparon mediante secuencia. Las medianas de las cargas virales de 54 muestras mostraron diferencias entre los métodos ($p < 0.0001$). El 52 % de las muestras presentó diferencias en la cuantificación de la carga viral > 0.5 log UI/mL. El coeficiente de Lin y el método de Bland Altman mostraron una pobre concordancia. Los valores de carga viral para los recombinantes BGs fueron diferentes entre los métodos ($p < 0.0091$) y fueron menores con el sistema EasyQ®. El método EasyQ® subcuantifica los recombinantes CRF_BGs cubanos, lo que limita el empleo de este ensayo para el seguimiento clínico de los pacientes infectados con esta variante viral. Por lo tanto consideramos que no deben emplearse indistintamente los ensayos de carga viral estudiados.

Palabras clave: Carga viral, subtipos de VIH-1, NucliSens® EasyQ® HIV-1, COBAS® TaqMan® 48 HIV-1

How to cite (Vancouver style):
Pérez L, Vélez S, Díaz HM, Pintos Y, Machado LY, Kourí V, *et al*. Comparison of NucliSens® EasyQ® HIV-1 v2.0 and COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 commercial systems for the determination of viral load of different HIV-1 genetic variants in Cuban patients. Biotecnol Apl. 2019;36(3):3221-6.

Introduction

The human acquired immunodeficiency syndrome (AIDS) was first described in 1981 by the Centers for Disease Control in US, and, since then, it has been a significant human health problem worldwide [1].

The precision and reproducibility of diagnostic tests to determine the RNA levels in plasma of its causing agent, the human immunodeficiency virus type 1 (HIV-1), and the absolute count of CD4+ T cells, are key elements for the follow up of infected individuals and clinical decision criteria [2, 3]. The determination of the viral load is used to evaluate viral infectivity of an individual [4], the risks for the progression of the disease [5], to supervise the response to the highly active antiretroviral therapy [6] and to evaluate the possible emergence of viral resistance [7]. In fact, HAART is defined as the suppression of plasma viremia based in two sequential quantitative determinations of plasma HIV-1 RNA in a six-month interval, which would be below the quantification limit of the approved VL [8].

There are several assays currently available for the detection of VL in plasma. The main differences rely on the formats, duration of testing and the capacity for sample processing. All the techniques

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detect and quantify mainly HIV-1 subtype B and other circulating subtypes (A, C, D, F, G). Tests marketed by Abbott and the COBAS® Ampliprep/COBAS® Taqman® 48 test from Roche actually quantify RNA from HIV-1 subtype O. None of these tests quantify HIV-2 [9].

Since HIV-1 strains diverge within a given geographic area, as well as worldwide, it will be increasingly important for clinical laboratories to establish which is the viral genotype leading the infection in the population. Patients with documented infection of non-B subtypes, as well as those with extremely divergent subtype B strains, could require HIV-1 VL tests in a different diagnosis platform if their results are discordant to their clinical state [10, 11].

In this sense, the studies carried out on the Cuban epidemic have identified subtypes A, B, C, D, F, G, H, CRF01_AE, CRF02_AG, complex circulating recombinant forms as CRF18_cpx, CRF19_cpx and CRF_BGs, and unique recombinants emerging from circulating subtypes [12, 13]. It is known that some of these subtypes were acquired in Africa during the 1970's and 1980's decades [14, 15].

In Cuba, two main commercial systems have been used for determining the HIV-1 VL: NucliSENS® EasyQ® HIV-1 version 2.0 (EQ) from Biomerieux, which was implemented in 2001, and the COBAS® Ampliprep/COBAS® Taqman® 48 version 2.0 (CAP/CTM), from Roche, since 2009.

In the clinic, both methods have been applied indistinctly to evaluate the state of the patient and the response to HAART. However, a study is missing to evaluate the agreement between both methods for determining VL in the population of individuals infected with the HIV-1 variants circulating in the country.

Therefore, in this work, the NucliSENS® EasyQ® HIV-1 version 2.0 (EQ) from Biomerieux and the COBAS® Ampliprep/COBAS® Taqman® 48 version 2.0 (CAB/CTM) from Roche were compared, and their performance evaluated against the HIV-1 variants circulating in Cuba.

Materials and methods

Study design and samples

An analytical study of a transversal cohort was done with plasma samples of patients infected with HIV-1 in the period from September 29th, 2015, to April 12th, 2016. The patients sample was formed by all the patients attending the Infectology Service at the Clinical Surgical Hospital Hermanos Ameijeiras, in Havana, Cuba, for having their routine clinical VL testing.

Up to 109 patients were included in the study, with 43 out of 54 (79.7 %) male, 23 (43.6 %) under ART, 39 (72.2 %) asymptomatic at the time of sampling, and 13 (24 %) classified as AIDS. Only two patients presented acute infection at sampling (3.7 %). The median value of CD4+ T cells was 295 cells/mm^3. Samples having undetectable VL values by one or both methods tested were regarded as exclusion criteria.

The study was subjected to the approval and it as approved by the Ethics Committee and the and the Institutional Scientific Committee of the Tropical Medicine Institute Pedro Kourí, in Havana, Cuba. The study complied with the ethics regulations of the Declaration of Helsinki on processing of human samples and studies with human subjects, and a written informed consent was signed by all the patients included in the study.

Specimens

Venous blood samples of 20 mL each were extracted in ethylenediaminetetraacetic acid under sterile conditions. Blood was transported frozen under optimal conditions and complying with the biosafety regulations to the Cuban AIDS Reference and Research Laboratory (LISIDA). The samples were centrifuged at 1500 g for 10 min and plasma extracted. Plasma was then split into five 1-mL aliquots and immediately frozen at –80 ºC until use. Then, three aliquots per patient were transported frozen to the Microbiology and Infectious Transmissible Diseases labs at the Virology Department of the IPK. One milliliter of plasma was processed per patient and RNA extracted for VL determinations by the NucliSENS® EasyQ® HIV-1 v2.0 (http://www.biomerieux-diagnostics.com) and the COBAS® AmpliPrep/COBAS® TaqMan® 48 (http://molecular.roche.com). Another milliliter of plasma was processed for the manual extraction of RNA and HIV-1 subtype identification according to manufacturer's instructions [16].

Viral load determinations

COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1

The COBAS® TaqMan HIV-1 test supports the quantitative detection of HIV-1 RNA from HIV-1 patients at the amplification range of 20-10 000 000 copies/mL [17]. This version employs primers flanking the gag gene and the long-term repeat (LTR) regions.

NucliSENS® EasyQ® v.2.0

This test supports the direct quantitation of HIV-1 RNA from plasma samples and dry blood drops on filter paper, in the range 10-10 000 000 copies/mL for viruses of clade M viruses (A-H and J) and recombinants CRF01_AE and CRF02_AG. It amplifies and quantifies in real time HIV-1 RNA from plasma samples and dry blood drops in filter paper from viral RNA. It is amplified by an isothermal amplification system designed by NASBA [18].

Genotypic resistance assay

HIV-1 was genotyped in 1 mL blood plasma centrifuged at 14 000 rpm for 1 h and the pellet resuspended in 140 µL with the remaining fluid. RNA was extracted using the QIAamp Viral RNA Kit (Qiagen, Germany), following the manufacturer's recommendations. The pol gene was reverse-transcribed, amplified and bidirectionally sequenced using the previously mentioned technology. This technique requires a viral load higher than 1000 copies/mL [16].

Each consensus sequence was obtained from the HIV-1 sample, sent to the Rega Subtyping database tool version 3 (http://regatools.med.kuleuven.be/typing/v3/hiv/typingtool), which supports HIV-1 determination automatically.

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Statistical analysis

A database was structured with some clinical features (infection status, CD4+ lymphocyte counts, viral load and antiviral treatment) and epidemiological features (sex and sexual preference), obtained from participants in the study. All the previously received information was obtained from the SIDATRAT Database [19] and the clinical history of patients preserved at the Department's archive and medical records at the IPK.

Statistical packages SPSS® v21, Epidat v3.1 and GraphPad prism v5.0.1 were used. Data were normalized according to D'Agustino Pearson and descriptive statistical measures were calculated. The Wilcoxon's test was run and results regarded as significant for $p < 0.005$. The agreement between both assays was determined by the Bland-Altman method, the correlation coefficient calculated and the Lin agreement score.

The variation coefficient values were calculated for the NucliSENS® EasyQ® and the COBAS® AmpliPrep/COBAS® TaqMan® 48 assays, the results in copies/mL been converted into international units (IU) to match the results and its comparison and then, expressed in log 10 scale (IU/mL). For the NucliSENS® EasyQ®, VL were transformed to IU/mL by dividing the values in copies/mL by 0.48 [20]. Meanwhile, the COBAS® AmpliPrep/COBAS® TaqMan® 48 VL values were multiplied in copies/mL by 1.67 [21].

The frequency distributions were obtained by both methods according to their concentration ranges. The VL ranges were: < 40, 41-1000, 1001-10 000, 10 001-100 000 and > 100 000 IU/mL.

Results

Comparison of HIV-1 VL values of patients included in the study by both commercial methods in Cuba

Of the 109 patients included in the research, 54 plasma samples were VL detectable by both methods. The rest of patients (55) were below the detection limit in both assays, declared as undetectable, and excluded from the analysis.

The VL median was 4.9 log IU/mL according to the CAP/CTM, higher than the EQ index of 4.5 log IU/mL. According to the non-parametric Wilcoxon's test, significant differences between both VLs were quantified by both methods ($p < 0.0001$).

As shown in figure 1, major discordances were found fundamentally in the VL ranges lower than 3 log IU/mL and at 5-7 log IU/mL (lower-upper limits). At the intermediate range (3-5 log IU/mL), VL values were similar between both assays, thereby showing a lower data dispersion.

Since both methods differ in the lower detection limit (see Materials and methods), the minimal value was set to 40 IU/mL. Then, VLs were divided in intervals and the respective frequencies of agreement calculated, in order to identify those showing the highest differences.

The frequencies were obtained at the intervals shown in table 1. Major discrepancies were detected at CV intervals of 10,001 and 100,000, and more than

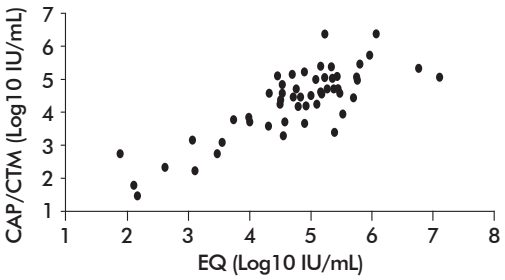

Figure 1. Dispersion diagram of HIV-1 VL (Log10 IU/mL) quantified in 52 samples of plasma from Cuban patients by the COBAS® AmpliPrep/COBAS® TaqMan® 48 (CAP/CTM) and the NucliSENS® EasyQ® (EQ).

Table 1. Viral load ranges of the HIV-1 cases detected with the NucliSENS® EasyQ® HIV-1 v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 commercial systems*

	VL intervals by each method	≤ 40 (%)	41-1000 (%)	1001-10 000 (%)	10 001-100 000 (%)	> 100 000 (%)	Total (n)
EQ(IU/mL)	≤ 40 (%)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	1 (1.8)
	41-1000 (%)	0 (0)	3 (5.5)	2 (3.7)	0 (0)	0 (0)	5 (9.2)
	1001-10 000 (%)	0 (0)	0 (0)	5 (9.2)	4 (7.4)	2 (3.7)	11 (3.7)
	10 001-100 000 (%)	0 (0)	0 (0)	0 (0.0)	11 (20.3)	11 (20.3)	22 (40.7)
	> 100 000 (%)	0 (0)	0 (0)	0 (0.0)	3 (5.5)	12 (22.0)	15 (27.7)
	Total n (%)	0 (0)	4 (7.4)	7 (12.9)	18 (33.3)	25 (46.2)	54 (100)

* Intervals were established according to the viral load (VL) values obtained by each method and transformed to IU/mL in 54 samples. Intervals were established according to the viral load (VL) values obtained by each method and transformed to IU/mL in 54 samples. VL values are highlighted in gray.

100,000 for CAP/CTM, with 4 (7.4 %) and 11 (20.3 %) values of VL dissenting values.

Frequencies are shown at intervals in Table 1. Major discrepancies were detected in the 10 001 to 100 000 and > 100 000 for CAP/CTM values, 4 (7.4 %) and 11 (20.3 %) dissenting, respectively. Both assays matched in 57.4 %, at the ranges highlighted in gray in Table 1.

The statistical correlation and the determination of the correlation coefficient have been shown insufficient to evaluate the agreement of both quantitative measures. Similarly, the Pearson's and Spearman's correlation coefficients are mistaken as concordance measurement [22, 23]. Thereby, the Lino's agreement coefficient was calculated and a Bland-Altman plotted as shown in figure 2. The 54 VLs showed differences of –0.416 and confidence limits for the 2.5 and 97.5 percentiles were –1.87 and 0.738, respectively. Major discrepancies were observed for means of 1 to 3 and 5 to 6 (2.4 and 3.4 log copies/mL). Differences for each sample measured by both methods in respect to every means as measured by both methods are also shown (Figure 2).

Due to the variability of VL tests and its possible transient and unexpected increase in patients, the last guidelines for the use of antiretroviral agents in adults and teenagers infected with HIV-1 define that a patient has an ongoing virological failure to the confirmed outcome of VL above 1000 copies/mL, in order to control the required treatment modifications [24].

Differences higher than 0.5 log between VL quantifications in one subject is regarded as clinically relevant and indicative for a change in the therapeutic conduct. Therefore, differences higher than 0.5 log

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are regarded as relevant, when assaying different VL methodologies [25, 26].

In this study, differences were calculated for both assays below 0.5 log, at the 0.51-1.0 log and above 1 log. The 48 % of viral load quantified by both methods showed differences below 0.5 log copies/mL [25]. Nevertheless, the highest differences between both methods were lower than 0.5 log copies/mL, which were accepted within the clinical tolerance range [25]. Nevertheless, differences between both methods higher than 0.5 log IU/mL in 52 % of cases.

VL values determined for the different methods according to identified viral subtypes

The HIV-1 viral genetic diversity represents a challenge for the development of tests able to efficiently detect and quantify all viral variants. In Europe and North America, most patients are infected with subtype B. In other parts of the world, infections by non-B subtypes in group M are more frequent or even the main [25, 26]. The determination of the viral subtype by a PCR-sequence in-house assay [16] to all the 54 plasma samples resulted in the amplification of 40 blood samples.

HIV-1 subtypes detected in the present study match with that reported in the Cuban epidemics so far. Subtype B was detected at a higher proportion in a 31.4 %, followed by recombinant BG (16.6 %) and the CRF19_cpx (12.9 %). It was also detected that other subtypes and recombinant forms, as A, G and CRF18_cpx (1.8 % each), as the C and the CRF06 (3.7 % each). [27-29].

It was not possible to obtain viral RNA by the in-house PCR test in 14 plasma tests. There were seven plasma tests by EQ and 5 by CAP/CTM, between the lower detection limit and the 1000 copies for the assays.

Commercial VL quantification tests are designed to detect HIV-1 subtype B, but it could also detect other subtypes or even other group M recombinant forms of higher efficiency. Some comparative studies have analyzed the agreement of the different commercial tests to detect and quantify VL in samples of patients infected with non-B HIV-1 subtypes. Therefore, differences were calculated above and below 0.5 log IU/mL between both assays for the viral subtypes detected, which could be observed in figure 3. Contingency tables were used to compare the most representative subtypes according to the described intervals and no significant differences were detected. Despite the lack of significance in the comparison, contingency tables were made to compare the most representative subtypes for the described intervals. Despite the lack of significance in the comparison, BG recombinants showed a difference of the 15.5 % above the 0.5 log, twice the differences for the same percentage observed below 0.5 log for the same recombinant between both methods.

In order to explore if any of the genetic forms were related with the differences in the quantification methods, VLs of most representative viral subtypes were compared (Subtypes B, CRF19_cpx and CRF_BGs), as well as the total VL of the 40 viral samples amplified by the in-house RT-PCR. As shown in figure 4, statistically significant differences were found for VL values between both assays for the 40 samples

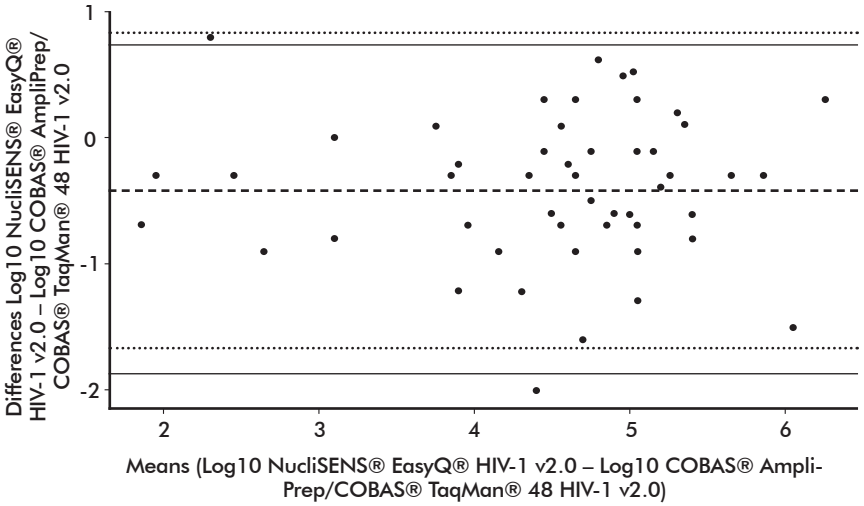

Figure 2. Concordance between COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 and NucliSENS® EasyQ® HIV-1 v2.0 commercial systems by the Bland-Altman method. The discontinuous line in the chart stands for the mean difference , corresponding to –0.416. Dotted lines stand for the upper (0.9965) and lower (–1.9580) limits; those values covering the 95 % confidence interval, for difference values when following a normal distribution. Continuous lines stand for empirical limits, considering that differences do not follow a normal distribution, in which case the upper and lower lines values are –1.87 and 0.735, respectively.

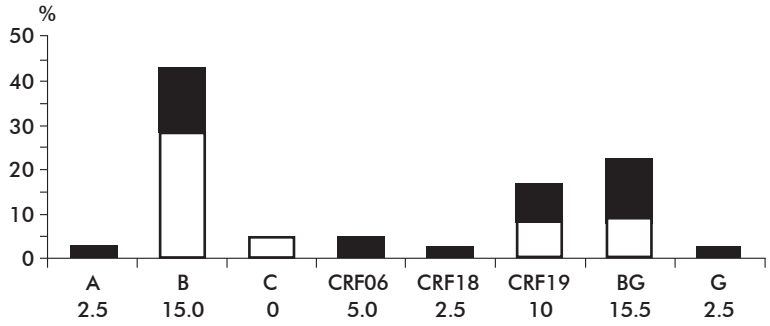

Figure 3. Percentual distribution of differences between COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 (Roche) and NucliSENS® EasyQ® HIV-1 v2.0 (Biomerieux) commercial systems (Log10 IU/mL) according to the different HIV-1 genetic variants detected by the in-house PCR-sequence method.

sequenced, coinciding with results of the 109 samples ($p < 0.0001$). Notably, such a difference was just significant for CRF_BGs (20, 23, 24; $p < 0.0279$), not for other subtypes.

Discussion

Discordant VL values grouped mainly on the ranges below 3 log IU/mL and in the 5-7 log interval, meanwhile the best correlation was observed in intermediate VL ranges (3 a 5 log IU/mL). This could be related to a better linearity in both methods in this interval. It is known that VL values at the low concentration ranges obtained by RT-PCR assays could show discrepancies among methodologies [4, 5]. Most studies have focused on the differences at the low concentration ranges and the lower detection limit. Nevertheless, discrepancies have been described in the highest concentration ranges (3.4 to 5.7 log copies/mL) when comparing CAP/CTM c. 2.0 with the standard Cobas

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Amplicor® (Roche), and even differences higher than 1.72 log copies/mL were reported [30].

The results obtained are in agreement with those reported by Jaramillo *et al*. in 2012, when comparing discrepancies by VL intervals. These are similar to those with CAP/CTM with Versan and similar discrepancies were obtained at those intervals [31].

Solís *et al*. comment that the main sources of variability among VL assays include differences in the technological platform, inlet volume of plasma and the capacity to detect HIV-1 subtypes [32].

International comparative studies among the different marketed quantification tests have shown a high degree of agreement, contrary to present results. Major discrepancies found in the scientific literature are observed at low VL values and in the detection of Non-B subtype, coinciding with our results [33-36].

New standardized practices and guidelines are required to improve the methods of assay for HIV-1 methods evaluation. This is particularly relevant to the definition of study population, the algorithms for inclusion notification and the sample exclusion throughout the study, as well as the technical expertise and the use of adequate statistical methods [24].

VLs differences higher than 0.5 log were found in the 50 % of samples, contrasting with the rest of the revised literature. This can arise from viral variants described in Cuba (CRF_BGs [20, 23, 24], CRF19_cpx, CRF18_cpx and other URFs), which have not been evaluated when designing commercial VLs, its circulation almost entirely restricted to Cuba [27]. However, Katsoulidou *et al*. and Solís *et al*. showed results different from those obtained in this work. They quantify differences lower than 0.5 log copies/mL in 86 % of the samples studied [26, 37].

Some RNAs were not quantifiable by the conventional methods used in the study to measure viral load, and they were amplified by the in-house RT-PCR technique designed to detect viral resistance. It has been considered that polymorphisms at the joining sites of primers or probes could be the cause for VL under-quantification in individuals affected by those changes. This theory has been documented in the literature, since a single mutation being detected in a Cobas® TaqMan HIV-1 primer from Roche reduces the viral quantification in > 2 log, when compared to other quantification methods, including the Cobas® Amplicor Monitor [38]. In this sense, recent publications [9, 36] comparing Non-B quantification subtypes among Versant® assays (kPCR) and the Siemens bDNA, or between LCx with Abbott's RealTime® show a lower Versan quantification of ≥ 0.5 log in all non-B subtypes and > 0.8 log for G and H subtypes of Abbott's Real-Time®. This should be an aspect to be surveiled in the new HIV-1 quantification platforms.

Nevertheless, a number of samples were found able to be quantified by methods used in the study, while it was not possible to amplify RNA by the in-house methodology. The circulation of multiple subtypes in the same geographic area makes possible the generation of new forms, which could negatively influence PCR results. In HIV-1 epidemiological surveillance studies of HIV-1 strains circulating in Cuba, almost all the known viral subtypes circulating in Africa have shown the highest viral variability, as well as circulating recombinant forms generated locally together with multiple unique forms. In recent years, new recombinant forms and complex unique recombinants have been observed, the reason why this could have been influenced on the low amplification of the in-house method (PCR-sequence) [13, 27, 28].

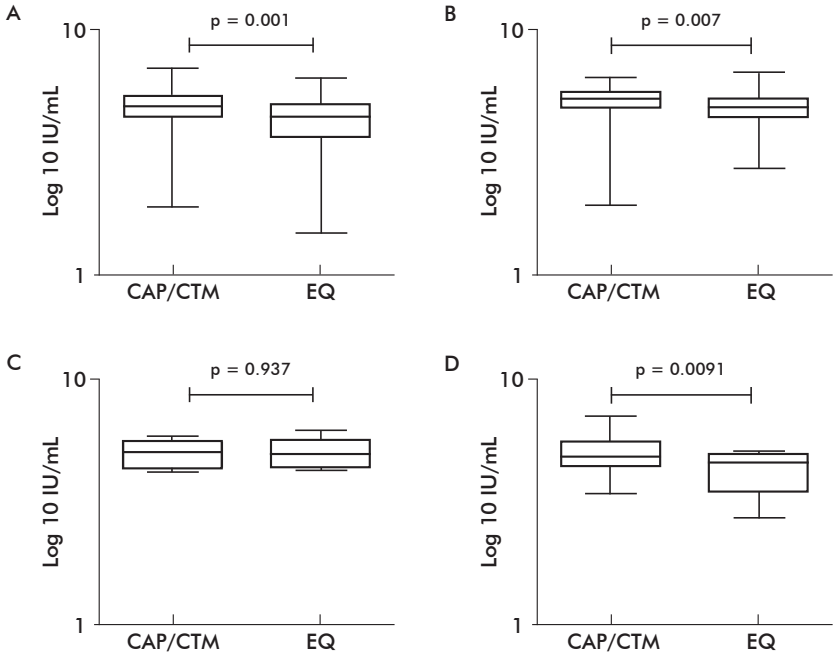

Figure 4. Comparison of viral loads quantified by COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 v2.0 (CAP/CTM; Roche) and NucliSENS® EasyQ® HIV-1 v2.0 (EQ; Biomerieux) commercial systems (Log10 IU/mL) of HIV-1 subtypes and its genetic variants. A) VL for all the samples tested (n = 54). B) VL for subtype B HIV-1 viruses (n = 17). C) VL for the HIV-1 virus genetic variant CRF_19 (n = 7). C) VL for the HIV-1 virus genetic variant BG (n = 9).

Patients with documented non-B subtype infection as well as those showing an extreme divergence within the subtype B could require HIV-1 VL testing with a different diagnostic system could require new HIV-1 VL tests with a platform different to that if they results seem to be discordant with its clinical presentation.

Discordant samples were re-assayed using a second HIV-1 VL test directed towards an alternative gag region (that is, gag, if a pol gene-based was primarily used, and viceversa). The periodical evaluation of the assays could be required to evaluate its performance to detect and quantify precisely divergent HIV-1 subtypes. They are aimed to provide an optimal performance for patients from either developed or low-income resource countries [39].

According to Church *et al*., EQ and bDNA methods showed the highest subquantification rates of > 1.0 log copies/mL, mainly for non-B HIV-1 subtypes. Performance significantly varied among HIV-1 VL platforms according to the subtype. HIV-1 viral diversity in the population subjected to the test should be considered to select the VL determination platform [39].

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Besides, Holguin *et al*. compared the yields of Versant v3.0 (bDNA), CAP/CPM and EQ tests in 83 samples of patients infected with HIV-1 non-B and recombinant subtypes. It was only possible to quantify 32 samples (58.2 %) by the three methods.

Summarizing, we recommend that VL results should have to be cautiously interpreted, when the monitoring is carried out in different assays and for the same patient. This is due to the variability of the test could increase the risk of over- or underestimation of results. According to pertinent studies [40], when VL shows discrepancies to the CD4+ cells or clinical samples, it is recommended that the HIV-1 RNA should be measured as alternative test [41].

The variability sources between VL quantification tests include not only the differences in the technological platform, but also the inlet plasma sample and the capacity to detect HIV-1 subtypes. Therefore, VL monitoring should be performed using the same technological platform, either to monitor individual patients as to guarantee an appropriate interpretation of VL changes, unless no clinically relevant differences have not been identified [32].

Conclusions

Overall, the EQ method detects lower VL levels than the CAP/CTM test, particularly in extreme ranges. The low concordance detected between both VL tests indicates that they would be regarded as equivalent. The detection of lower VL levels for the CRF_ BGs variants with the EQ method limits the use of this test for the follow up and the clinical management of infected patients carrying these recombinant strains.

Acknowledgements

The authors are grateful to all the patients who agreed to participate in the trial. The study funded by the World Fund to Fight Against AIDS, Malaria and Tuberculosis.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Received in Apr, 2019.
Accepted in June, 2019.