Performance evaluation of Human Immunodeficiency Virus Type 1 RNA Quantitative Diagnostic Kit assay

Avaliação de desempenho do ensaio do Kit de Diagnóstico Quantitativo de RNA do vírus da imunodeficiência humana tipo 1

Alicia Fleites Vázquez[1], *María Teresa Pérez Guevara*[1]

ABSTRACT

Introduction: Quantitative determination of human immunodeficiency virus (HIV) concentration is an important parameter for the prognosis and treatment of infected people, contributing to understanding the infection's pathogenesis. The quantitative real-time polymerase chain reaction (qPCR) methodology offers numerous advantages over traditional molecular methods for measuring HIV viral load. In Cuba, the HIV Type 1 RNA Quantitative Diagnostic Kit was developed as an additional option to the existing technology for HIV-1 RNA determination. **Objective:** This work aimed to evaluate the performance of the HIV Type 1 RNA Quantitative Diagnostic Kit (PCR - Fluorescence Probing) for quantitative detection of HIV-1 RNA in human samples. **Methods:** We evaluated human serum and plasma samples previously characterized by molecular techniques. Five panels of HIV positive and negative samples were used for studies of concordance, diagnostic sensitivity, clinical and analytical specificity, intra- and inter-assay precision, and linearity and robustness. We also evaluated an international standard for testing analytical sensitivity. **Results:** Concordance, diagnostic sensitivity, and specificities resulted in 100% reliability. Coefficients of variation of less than 20% and 17% were obtained in intra- and inter-assay precision, respectively. The analytical sensitivity was 32.4 IU/mL, inferior to that established by the producer (50 IU/mL). The assay showed linear behavior, and the robustness was confirmed by performing the technique by different operators in different equipment and laboratories. **Conclusion:** The results demonstrate the feasibility of using the HIV Type 1 RNA Quantitative Diagnostic Kit for the quantitative detection of HIV-1 RNA in human serum or plasma samples.

Keywords: HIV. PCR. RNA. Viral load.

RESUMO

Introdução: A determinação quantitativa da concentração do vírus da imunodeficiência humana (HIV) é um parâmetro importante para o prognóstico e tratamento de pessoas infectadas, contribuindo para compreensão da patogênese da infecção. A metodologia quantitativa da reação em cadeia da polimerase em tempo real (qPCR) oferece inúmeras vantagens sobre os métodos moleculares tradicionais na medição da carga viral do HIV. Em Cuba, o Kit de Diagnóstico Quantitativo de RNA do HIV Tipo 1 foi desenvolvido como uma opção adicional à tecnologia existente para a determinação do RNA do HIV-1. **Objetivo:** Este trabalho visa avaliar o desempenho do Kit de Diagnóstico Quantitativo de RNA do HIV Tipo 1 (PCR - Sondagem da Fluorescência) para detecção quantitativa do RNA do HIV-1 em amostras humanas. **Métodos:** Avaliamos amostras de soro e plasma humano previamente caracterizadas por técnicas moleculares. Cinco painéis de amostras positivas e negativas para HIV foram usados para estudos de concordância, sensibilidade diagnóstica, especificidade clínica e analítica, precisão intra e interensaio, além de linearidade e robustez. Também avaliamos um padrão internacional para testar a sensibilidade analítica. **Resultados:** Concordância, sensibilidade diagnóstica e especificidades resultaram em 100% de confiabilidade. Coeficientes de variação abaixo de 20% e 17% foram obtidos na precisão intra e interensaio, respectivamente. A sensibilidade analítica foi de 32,4 UI/mL, inferior à estabelecida pelo produtor (50 UI/mL). O ensaio apresentou comportamento linear, e a robustez foi confirmada pela execução da técnica por diferentes operadores em diferentes equipamentos e laboratórios. **Conclusão:** Os resultados demonstram a viabilidade do uso do Kit de Diagnóstico Quantitativo de RNA do HIV Tipo 1 para a detecção quantitativa de RNA do HIV-1 em amostras de soro ou plasma humano.

Palavras-chave: HIV. PCR. RNA. Carga viral.

INTRODUCTION

The human immunodeficiency virus (HIV) is a retrovirus of the family *Retroviridae,* genus *Lentivirus*, with a specific tropism for CD4+ cells, which it infects and destroys[1]. It is transmitted through the blood. It can also be transmitted sexually, by sharing needles or other intravenous utensils with infected people, from mother to child at birth, or through breast milk[1,2].

Patients with HIV who are adequately treated do not develop acquired immunodeficiency syndrome (AIDS)[1], a condition that leads to chronic deterioration of the immune system due to a progressive loss of CD4 lymphocytes with the appearance of opportunistic diseases, additional infections, and tumors with serious consequences, that can be fatal to the individual[3]. The quantitative determination of HIV concentration in peripheral blood has contributed significantly to the understanding of the infection's pathogenesis[4]. Molecular diagnosis, as a novel *in vitro* diagnostic technique, is of great medical value in the identification of diseases and the development of treatment and monitoring programs, as well as complementing traditional *in vitro* diagnostic methods[5].

A variety of commercial tests are currently available to quantify HIV viral load, with differences in sensitivity, linearity, virus genome target sites, and amplification methods. The most recent tests are based on quantitative real-time polymerase chain reaction (qPCR) methodology, which has been widely adopted by laboratories due to its higher sensitivity, wider dynamic range, high degree of automation, and greater ability to quantify different types and subtypes of HIV[6,7].

[1]AIDS Research Laboratory – San José de las Lajas, Mayabeque, Cuba. E-mails: alicia.fleites@yahoo.com; dc_calidad@unicom.co.cu

HIV Type 1 RNA Quantitative Diagnostic Kit (PCR - Fluorescence Probing) (Sansure Biotech Inc.) is a fluorescent qPCR for the quantification of HIV-1 RNA. The detection result can be used to aid in the diagnosis of HIV-1 infection as well as for monitoring the therapeutic effect of antiviral drugs in infected patients[8]. This diagnostic test would be another powerful tool in monitoring people living with HIV.

Registration with the regulatory body is a prerequisite for marketing authorization of this product in Cuba. The Center for State Control of Medicines, Equipment and Medical Devices (CECMED) has the mission to guarantee the protection of public health by ensuring, through a health regulation and control system, that medicines and diagnostics available for human use, whether imported or domestically manufactured, have the required safety, efficacy, and quality[9]. Regulation D 08-2013 establishes the requirements for the Health Register of Diagnosticians where the evaluation of the performance of the products used in the screening and confirmation of risk class D microorganisms is carried out; Regulation 47-2007 in turn, establishes the requirements for this evaluation[10,11]. In this sense, the AIDS Research Laboratory (LISIDA) is the laboratory designated to carry out these studies on human retroviruses[11].

OBJECTIVE

This work aimed to evaluate the performance of the HIV Type 1 RNA Quantitative Diagnostic Kit (PCR – Fluorescence Probing) for the quantitative detection of HIV-1 RNA in human serum and plasma samples.

METHODS

The tests were performed following the manufacturer's recommended instructions for use[8] and the standards established by the national regulatory authority[10,11] when carrying out the tests. The COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Test version 2.0 was used as the reference diagnostic tool, based on the conversion of copies per milliliter of peripheral blood to international units (IU) multiplied by the conversion factor of 1.67.

Evaluated essay

HIV Type 1 RNA Quantitative Diagnostic KIT (PCR – Fluorescence Probing) (Sansure Biotech Inc.) uses a fluorescent qPCR technique to quantify HIV-1 RNA in human serum and plasma samples. It is validated by the manufacturer in qPCR instruments based on plate amplification and detection systems. It has a detection limit of 50 IU/mL and a linear range from $5x10^{1}$ IU/mL to $1x10^{8}$ IU/mL[8].

Reference test

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 is an *in vitro* nucleic acid amplification test for the quantification of HIV-1 RNA in human plasma using the COBAS® AmpliPrep Instrument for automatic sample processing and COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automatic amplification and detection. The test allows quantification of HIV-1 RNA in the range of 20 to 10,000,000 copies/mL[12].

Samples

Five panels of clinical samples were used, previously characterized by the LISIDA Molecular Biology Laboratory and a panel of dilutions of an International Standard (IS). The panels were formed as follows:

Panel 1: Ten HIV-1 negative plasma samples;

Panel 2: Six plasma samples from HIV-1 positive patients, with different viral load levels (<20 IU/mL to 1.85E+05 IU/mL);

Panel 3: Two samples with sequences of HIV-2, human T-cell lymphotropic virus (HTLV-1), hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* and from seronegative pregnant women for a total of 12 HIV-1 negative plasma samples, with the possibility of producing interference or cross-reactivity;

Panel 4: Ten HIV-1 positive plasma samples, with different subtypes, circulating recombinant forms (CRFs), and unique recombinant forms (URFs), circulating in Cuba (CRF 18_cpx, CRF 19_cpx, CRF 20_BG, CRF 24_BG, URF BG, URF BD, subtype A, subtype B);

Panel 5: Four dilution samples of IS WHO/NIBSC code 10/152: 125 IU/mL; 62.5 IU/mL; 31.2 IU/mL; and 15.6 IU/mL. The IS was reconstituted with 1 mL of RNA-free water to obtain a final concentration of 185,000 IU/mL; and then diluted with HBV, HCV, and HIV-1 negative human serum to obtain the desired HIV-1 RNA concentrations; and

Panel 6: Five HIV-1 positive human serum samples.

Variables

We calculated the percentage of concordance between the HIV Type 1 RNA Quantitative Diagnostic Kit (PCR – Fluorescence Probing) and the COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Test version 2.0 system, using the samples corresponding to panels 1, 2, 4, and 6.

Samples from panel 1 were analyzed to determine clinical specificity, while those from panel 3 were used to determine analytical specificity (selectivity), taking into account that positive samples should not be obtained in any case.

The results of the positive samples in panels 2, 4, and 6 were considered in the evaluation of diagnostic sensitivity. The acceptance criterion was that no negative results should be obtained.

Samples corresponding to panel 5 were included in the analysis of analytical sensitivity. The limit of detection (LOD) at the 95th percentile was determined applying the PROBIT method in the Minitab Release 14 program.

For linearity, ten replicates of the IS dilutions were evaluated, and Pearson correlation coefficient (r) and coefficient of determination (R^2) were calculated through the Origin 9.0 program.

To study the precision, three tests were performed on three different days, using five replicates of the sample belonging to panel 2 with a viral load of 5.06E+04 IU/mL, for a total of 15 replicates evaluated. For the inter-assay precision, changes were made in the operator, equipment, and laboratories used; the first two tests were performed at LISIDA with the Rotor-Gene Q equipment and the third at the Immunoassay Center with SLAN-96P equipment. For the analysis of intra-assay precision, the results obtained from replicates of the samples in each assay were analyzed. The mean, standard deviation (SD)

and coefficient of variation (CV) of HIV-1 RNA concentration (IU/mL) were calculated.

The assays for this diagnostic are validated by the manufacturer in equipment with a system for amplifying and detecting system for genetic material in plates, such as the SLAN-96P (Sansure Biotech Inc.). In this study, the Rotor-Gene Q (QIAGEN) was used, an instrument with a technology for amplification and detection of genetic material in a carousel[13], which is not one of the validated technologies. The robustness of the diagnostician was confirmed by comparing the results obtained with the samples from panel 5 when performing the technique using the Rotor-Gene Q. In addition, positive and negative samples were alternated in the same run to determine if cross-contamination between samples occurred during the extraction, amplification, and detection procedures.

RESULTS

The percentage of concordance between the two tests was 100% for samples from panels 1, 4, and 6. Samples from panel 2 were amplified except for the sample with a viral load <20 IU/mL, which did not amplify for either system, resulting in an overall concordance of 100%.

The samples from the first panel were negative in the assays, for 100% clinical specificity. No cross-reactivity or interference was found with samples in panel 3, resulting in 100% analytical specificity.

Positive results were obtained from all samples in panel 4. The same results were obtained from panel 6 samples. Of the samples in panel 2, the one with <20 IU/mL did not amplify; however, as its value was below the detection limit set by the manufacturer for the HIV Type 1 RNA Quantitative Diagnostic Kit, it was not included in the calculation of diagnostic sensitivity, which was 100%.

When 12 replicates of the IS concentrations were tested, 100% positivity was achieved with 125 IU/mL and 62.5 IU/mL concentrations. The 31.2 IU/mL and 15.6 IU/mL replicates yielded 91.7% as one did not amplify. The 95% limit of detection of the assay calculated by PROBIT analysis was 32.4 IU/mL (±15).

The assay showed linear behavior in the range evaluated with R^2=0.99, a maximum error of approximately 0.08, and an equation y=0.98x+0.02. For the samples evaluated, r=0.99.

The intra-assay precision tests showed CVs<20% for HIV-1 RNA concentration and <2% for $\log_{10}$ values (Table 1). For the inter-assay precision test, CV=~17% for concentration and <2% for $\log_{10}$ values.

In the robustness evaluation, we did not observe any cross-contamination between samples during the testing procedures, as 100% of the positive and negative samples were correctly identified. Using the Rotor-Gene Q instrument, we obtained a limit of detection (95% LOD) of 44 IU/mL.

DISCUSSION

The HIV/AIDS pandemic remains a global public health problem of unprecedented proportions. To facilitate diagnosis, expand access to antiretroviral drugs, and reduce mortality due to AIDS, it is necessary to optimize viral load quantification techniques, timely treatment and monitoring of patient progress[14,15].

HIV viral load quantification assays based on qPCR methodology offer numerous advantages over traditional molecular methods, including complete automation, reduction in the number of steps, the time required to perform them, and the ability to reduce contamination during the assay process, increased sensitivity through the use of fluorescence in detection, and a greater and wider dynamic range[14,16,17].

Commercial assays used for the quantification of HIV plasma RNA must have a good correlation with each other in order to guide appropriate therapeutic management[14,18]. In this study, 100% concordance was achieved between the HIV Type 1 RNA Quantitative Diagnostic Kit assay and the reference assay.

The results met the stated acceptance criteria for specificity and sensitivity. Specific HIV-1 sequences were detected and there was no interference or cross-reactivity with the samples tested. The linear equation showed good results with an R^2 close to 1; therefore, it can be said that there is a linear association with the IS, and it is possible that knowing one of the values (expected) predicts the result (observed) perfectly[19]. The correlation coefficient obtained is above the range of 0.950 to 0.970 reported by other authors in HIV RNA quantification assays[19-21], and given its value, it can be concluded that there is a strong, excellent positive correlation[22].

In the present study, good precision was obtained in both inter- and intra-assays. The CV values were found to be below those recommended for nucleic acid quantification assays[18]. The results provide diagnostic confidence as the variability in the assay is not confused with a change in the patient's viral load[23,24].

The robustness results show that it is possible to use the Rotor-Gene Q instrument, although it is not one of the instruments validated by the manufacturer. The LOD obtained (44 IU/mL) is close to that described by the manufacturer for the validated instruments (50 IU/mL). Therefore, it is acceptable to perform the determinations in carousel amplification and detection systems in addition to plate technology.

Strengths

The study was carried out at LISIDA, designated by the Cuban regulatory authority for the evaluation of human retroviruses, which guarantees the validity and quality of the results. The introduction of

Table 1. Intra-assay precision when using the Human Immunodeficiency Virus Type 1 RNA Quantitative Diagnostic Kit (PCR – fluorescence probing) diagnostic device.

	HIV-1 RNA concentration (IU/mL)			$\log_{10}$ HIV-1 RNA concentration		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Test 1	2.12E+04	3,162.73	14.9	4.32	0.06	1.4
Test 2	1.69E+04	1,681.36	9.9	4.23	0.04	1.0
Test 3	1.55E+04	2,891.20	18.6	4.19	0.07	1.8

PCR: polymerase chain reaction; HIV: human immunodeficiency virus; SD: standard deviation; CV: coefficient of variation.

a new real-time PCR kit for the quantification of HIV-1 represents a valuable tool for the diagnosis and monitoring of patients, contributing to public health by improving care for people living with HIV. This process has ensured compliance with the safety and efficacy standards established by the CECMED, which supports its marketing license.

Limitations

One of the main limitations of the study is that the real-time PCR kit requires a manual procedure, demanding highly qualified and trained personnel for its correct execution. It is essential to ensure compliance with good laboratory practices, thus minimizing the risk of contamination, invalidation, and erroneous results. In addition, the dependence on specific equipment may restrict the accessibility of the kit in certain laboratories, especially in regions with limited resources.

CONCLUSION

The results demonstrate the feasibility of using the HIV Type 1 RNA Quantitative Diagnostic Kit for the quantitative detection of HIV-1 RNA in human serum or plasma specimens and its potential use on platforms other than those validated by the manufacturer.

ACKNOWLEDGEMENTS

The authors are grateful to the Immunoassay Center for allowing us to perform part of the tests in their facilities and to all the people who cooperated in carrying out this study.

Approval by the Human Research Ethics Committee

The protocol was reviewed and approved by the AIDS Research Laboratory (LISIDA) ethics committee in May 2022 before initiating any procedure. The human serum and plasma samples used were remnants from existing samples at LISIDA for HIV diagnosis. These samples are coded upon receipt in the laboratory and recorded for conformation of the study panels. No personal information about the patients was used.

Participation of each author

AFV: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MTPG: Conceptualization, Project administration, Supervision, Writing – review & editing.

Funding

The authors declare no financial support.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Abdulghani N, González E, Manzardo C, Casanova JM, Pericás JM. Infección por el virus de la inmunodeficiencia humana (VIH). Síndrome de inmunodeficiencia adquirida. FMC. 2020;27(3 Suppl 1):63-74. https://doi.org/10.1016/j.fmc.2020.03.008
2. Morán Arribas M, Rivero A, Fernández E, Poveda T, Caylá JA. Magnitud de la infección por VIH, poblaciones vulnerables y barreras de acceso a la atención sanitaria. Enferm Infecc Microbiol Clin (Engl Ed). 2018;36(Suppl 1):3-9. https://doi.org/10.1016/S0213-005X(18)30239-8
3. Bell Pérez K, Coronado Rojas N, Polanco García BP, Laugart Wilson A, Campello Cala MP. Modificación de conocimientos acerca de VIH/sida en trabajadores de una fábrica de Guantánamo. Revista Información Científica. 2016;95(5):731-8.
4. Adams P, Vancutsem E, Nicolaizeau C, Servais JY, Piérard D, François JH, et al. Multicenter evaluation of the cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800 system for the quantification of HIV-1 plasma viral load. J Clin Virol. 2019;114:43-9. https://doi.org/10.1016/j.jcv.2019.03.008
5. Shao J, Lin C. Important companies. In: Song H, Yao J, eds. Vitro diagnostic industry in China. Singapore: Springer; 2021. p. 97-105. https://doi.org/10.1007/978-981-16-2316-5_10
6. Salimnia H, Moore EC, Crane LR, Macarthur RD, Fairfax MR. Discordance between viral loads determined by Roche COBAS AMPLICOR human immunodeficiency virus type 1 monitor (version 1.5) Standard and ultrasensitive assays caused by freezing patient plasma in centrifuged becton-dickinson vacutainer brand plasma preparation tubes. J Clin Microbiol. 2005;43(9):4635-9. https://doi.org/10.1128/JCM.43.9.4635-4639.2005
7. Tamay de Dios L, Ibarra C, Velasquillo C. Fundamentos de la reacción en cadena de la polimerasa (PCR) y de la PCR en tiempo real. Investigación en Discapacidad. 2013;2(2):70-8.
8. Sansure. S3046E HIV-1 – Human Immunodeficiency Virus Type 1 RNA Quantitative Fluorescence Diagnostic Kit [Internet]. 2022 [cited on 2022 May 12]. Available from: https://www.sansureglobal.com/product/s3046e-hiv-1/
9. Portuondo Sánchez CL, Alvarez Hernandez Y. Curso virtual regional sobre regulaciones de dispositivos médicos en la autoridad reguladora CECMED [Internet]. Cuba Salud; 2018 [cited on 2022 Sept 26]. Available from: http://www.convencionsalud2018.sld.cu/index.php/connvencionsalud/2018/paper/viewPaper/1331
10. República de Cuba. Ministerio de Salud Pública. Centro para El Control Estatal de Medicamentos, Equipos y Dispositivos Médicos. Regulación D-08-13: Requisitos para la autorización de comercialización de los diagnosticadores [Internet]. 2012 [cited on 2022 Sept 26]. Available from: https://www.cecmed.cu/sites/default/files/adjuntos/Reglamentacion/reg_d_08-13red.pdf
11. República de Cuba. Ministerio de Salud Pública. Centro para El Control Estatal de Medicamentos, Equipos y Dispositivos Médicos. Regulación No. 47-2007: Requisitos para la evaluación del desempeño de los diagnosticadores. [Internet]. 2007 [cited on 2022 Sept 26]. Available from: https://www.cecmed.cu/sites/default/files/adjuntos/Reglamentacion/Reg_47-07.pdf
12. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test [Internet]. ROCHE; 2007. [cited on 2023 Jan 18]. Available from: https://www.fda.gov/media/73824/download
13. QIAGEN. Rotor-Gene Q user manual [Internet]. 2022 [cited on 2023 Jan 18]. Available from: https://www.qiagen.com/no/resources/resourcedetail?id=26e14396-0e26-4faa-823a-c07e23780527&lang=en
14. Monteverdi ML, Cortiñas E, Lucca A. Verificación del desempeño analítico del método de cuantificación de carga viral de VIH M2000 ABBOTT-RT y comparación con el método COBAS TAQMAN VIH 2.0 ROCHE [Internet]. Bioquinforma Digital. 2021;1-4 [cited on 2023 Ago 08]. Available from: https://cobico.com.ar/wp-content/archivos/2022/01/VERIFICACI%C3%93N-DEL-DESEMPE%C3%91O-ANAL%C3%8DTICO.pdf
15. Alonso Carballo J, Gómez Gutiérrez M, Pérez Rodríguez A, de la Fe Mora Y. Caracterización clínico-epidemiológica de los pacientes con VIH/SIDA que padecieron COVID-19 en 2021 en Florida – Cuba. Revista Médica Sinergia. 2022;7(6):e824. https://doi.org/10.31434/rms.v7i6.824

16. Chevaliez S, Bouvier-Alias M, Pawlotsky JM. Performance of the Abbott real-time PCR assay using m2000sp and m2000rt for hepatitis C virus RNA quantification. J Clin Microbiol. 2009;47(6):1726-32. https://doi.org/10.1128/JCM.01300-08
17. NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements. 2002;19(3):1-46. PMID: 14768714.
18. Ramírez JD, Gudiño EA, Aguilar MS, D'Angelo P, Ariza H, Reyes CDV, et al. Estudio comparativo entre dos técnicas comerciales para la cuantificación de carga viral plasmática en pacientes infectados por el virus de inmunodeficiencia humana tipo 1. Rev Inst Nac Hig. 2016;47(1-2):27-42.
19. Rodriguez Lay LA, Montalvo MC, Bello Corredor M, López Hernández D, Martinez Montesino Y, Marrero Sanchez B, et al. Evaluación de una reacción en cadena de la polimerasa en tiempo real simple y rápida para la cuantificación del ADN del virus de la hepatitis B. Rev Cubana Med Trop. 2020;72(2):e522.
20. Pan P, Tao X, Zhang Q, Xing W, Sun X, Pei L, et al. Clinical comparison of branched DNA and reverse transcriptase-PCR and nucleic acid sequence-based amplification assay for the quantitation of circulating recombinant form_BC HIV-1 RNA in plasma. AIDS. 2007;21(Suppl 8):S27-32. https://doi.org/10.1097/01.aids.0000304693.16767.66
21. Schumacher W, Frick E, Kauselmann M, Maier-Hoyle V, van der Vliet R, Babiel R. Fully automated quantification of human immunodeficiency virus (HIV) type 1 RNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol. 2007;38(4):304-12. https://doi.org/10.1016/j.jcv.2006.12.022
22. Martínez Ortega RM, Tuya Pendás LC, Martínez Ortega M, Pérez Abreu A, Cánovas AM. El coeficiente de correlación de los rangos de Spearman caracterización. Rev Habanera Cienc Méd. 2009;8(2).
23. Barriga AG, Solis TM, Aceves RA. Estudio comparativo de dos pruebas para determinar carga viral para VIH-1 y VHC basadas en RT-PCR de tiempo real. Rev Mex Patol Clín Med Lab. 2009;56(4):247-56.
24. Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev. 2010;23(3):550-76. https://doi.org/10.1128/CMR.00074-09

Address for correspondence
ALICIA FLEITES VÁZQUEZ
National Highway and Tapaste Road Km 32[1/2].
San José de las Lajas, Mayabeque, Cuba.
E-mail: alicia.fleites@yahoo.com

Received on: 12/03/2024
Approved on: 03/26/2025