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Challenges and Pragmatic Solutions for Assessing the Reliability of HIV-1 Viral Load Monitoring in Resource-Constrained Settings

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Keywords
HIV-1 · RT-PCR · Quality control · Viral load · Resource-constrained settings

Abstract
Introduction: HIV-1 RNA detection is the most reliable method for monitoring treatment response among people living with HIV. Effective quality control measures that include internal quality control (IQC) are challenging in resource-constrained settings. ***Methods:*** We ascertained the utility of the kit low positive control (LPC) as an effective IQC to monitor the reliability of the HIV-1 viral load assay. Variations in LPC values were measured for 390 different runs over 10 years (2011–2021) and compared to in-house IQC data using Levey-Jennings control chart. ***Results:*** Overall, the Levey-Jennings analysis showed minimal variation (±0.5 log) for both the LPC and IQC data. The mean LPC value for first 20 runs (20 days) was 2.91. The mean LPC value for the 390 runs comprising 35 different lots was 3.01 ± 0.1 log. ***Conclusion:*** Our decadal data reveal that Abbott RealTime HIV-1 assay (Abbott Molecular Inc., IL, USA) LPC exhibited no significant biological variation over 390 runs distributed over 10 years. Hence, assay LPC can supplant the IQC for monitoring assay trends as a stable and commutable material in resource-constrained settings.

Introduction

The burden of people living with HIV in India is about 2.4 million [1]. To bring all HIV-infected individuals under universal coverage of testing and treatment facilities, UNAIDS has made efforts to achieve an ambitious target of 95-95-95 by 2025 [2]. The National AIDS Control Organization (NACO) has made laudable efforts in comprehensively undertaking and strengthening the HIV viral load programme [3]. To achieve the final 95 (95% of the individuals on treatment should be virally suppressed) in the 95-95-95, NACO has scaled up its efforts in establishing a nationwide network of HIV-1 viral load testing facilities in 2019 through public and public-private partnerships [4]. Estimating HIV-1 RNA is the most reliable method for the early detection of treatment failure [5]. However, it is the responsibility of the individual laboratory to offer accurate and reliable results. Erroneous results may significantly impact the quality of patient care [6]. Studies have shown that following a laboratory error, 2.7–13% have suffered an adverse event, and the analytical errors in an assay were around 7–13% [7]. These errors can be detected and minimized using robust internal control, improving the quality of laboratory results and patient care [8]. Though the supporting agencies provide the analytical equipment, the components of quality assurance are not included in the

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laboratories' budget, especially in resource-constrained settings [9]. The paper aims to evaluate the use of low positive kit control (LPC) as a potential substitute for internal quality control (IQC) for monitoring the trend analysis of viral load testing in resource-constrained settings.

Materials and Methods

The evaluation was done at the Department of Clinical Virology, Christian Medical College, Vellore, India, which provides laboratory services to approximately 4,000 people living with HIV annually. Institutional Review Board (IRB) approval was obtained for the use of stored samples and blood bank samples for the preparation of IQC (IRB Minute No. 14461, dated February 23, 2022). For the publication of this work, a waiver was obtained in August 2023. Over 10 years (2011–2021), we performed around 390 HIV-1 viral load runs. The CE-approved Abbott RealTime HIV-1 assay (Abbott Molecular Des Plaines, IL, USA) was used for all 390 runs. This assay had three reagent kits: an HIV-1 amplification reagent kit, a control kit, and a calibrator kit. The primers included in the amplification reagent kit targeted the *pol integrase* region of HIV-1. The control kit provided by the manufacturer had three-level controls: negative control, low positive control, and high positive control. The assay was performed on the Abbott m2000sp and m2000rt platforms (Abbott Molecular Inc., IL, USA). A 24-format run of 18 patient samples, two negative controls (water and negative plasma), three kit controls (negative, low positive, and high positive), and one IQC were used for every viral load run. The viral load assays performed were completed as part of standard patient care. We compiled the LPC viral load values for all 390 runs, plotted the Levey-Jennings control chart, and compared them with the laboratory's IQC.

Preparation of IQC

The IQC was prepared from blood bank samples and stored residual diagnostic samples. Informed consent was obtained from the blood bank donors and patients who gave the blood samples for diagnostic tests to use the remaining samples for quality control and research after anonymizing the samples. Two different IQC batches were prepared and used in the study. The first IQC batch was prepared using samples with 3.45 log copies/mL of HIV-1 and was used for 119 viral load runs. The second batch of IQC prepared had a viral load copy number of log 3.64 copies/mL, which was used for 101 runs. The IQC values were selected based on the clinical decision-making cutoff value of 1,000 (3 log) copies/mL as per the WHO guideline [10]. The prepared IQC was aliquoted as 1 mL vials and stored in a −80°C freezer till further testing, and a single-use aliquot was used for each HIV-1 viral load run. Twenty aliquots of the newly prepared IQC were run in quadruples over 5 days. The IQC mean and range (±0.5 log) were calculated based on the viral load copies of the first twenty runs.

Statistical Analysis

The Levey-Jennings control chart was prepared using the MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

Results

The study accumulated 390 valid viral load runs over 10 years. We prepared a Levey-Jennings chart with the available data to check the manufacturer's low positive control performance for all 390 runs. Thirty-five different lot reagents of the LPC were used during the study duration. Each LPC has a fixed range as provided by the manufacturer; this range varies from one lot to another. For all 390 viral load runs, the LPC value was within their respective range. The mean of the first twenty viral load runs was 2.91 log copies/mL. The upper and lower control limits were set as 3.41 log copies/mL and 2.41 log copies/mL, respectively, based on a 0.5 log difference from the mean. Among the 35 different lots of LPC used, four lots (69 runs) had a higher range but within a 0.5 log difference (shown in Fig. 1a). Another Levey-Jennings chart for 321 runs was plotted, excluding the four lots (shown in Fig. 1b).

Internal Quality Control

The first batch IQC mean calculated was 3.44 log copies/mL; the upper and lower limits were 3.94 and 2.94 log copies/mL, respectively (shown in Fig. 2a). The IQCs of the 119 runs were within the range and were tested using 13 different lots of HIV-1 reagents. Similarly, the second batch of IQC (shown in Fig. 2b) had a mean of 3.68 with upper and lower limits of 4.18 and 3.18, respectively. The IQCs of the total 101 runs were within the range and tested using nine different lots of HIV-1 reagents.

Discussion

As per the ISO 15189:2022 standards for medical laboratories, clause 7.3.7.2, quality control procedures play a crucial role in achieving the intended quality of results; their primary purpose is to check the reliability of the analytical system [11]. Therefore, viral load laboratories with good quality assurance programmes use IQC in the examination phase of an assay to certify the acceptability of the results [12]. However, a viral load laboratory in resource-constrained settings faces many challenges. The lack of accessibility to HIV-positive plasma bags, maintenance of a −80°C freezer for the long-term storage of the IQC samples, lack of continuous power supply, and trained staff in quality control measures majorly influence the outcome of the laboratory results [9]. Furthermore, it incurs a financial constraint on the laboratory's budgets [13], so quality control is not given high priority. The absence of robust quality control

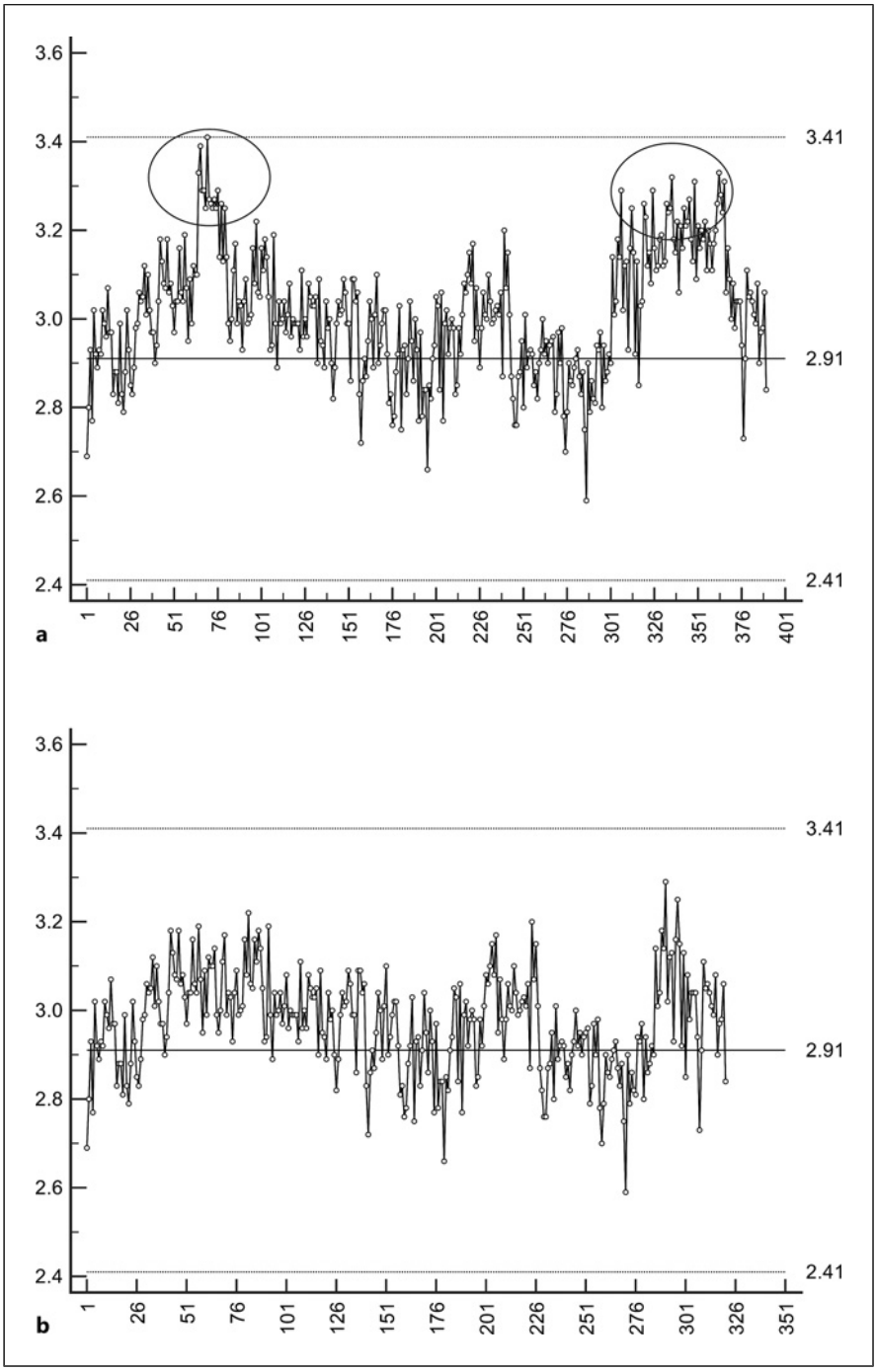

Fig. 1. a Levey-Jennings control chart of assay LPC of all 390 viral load runs (2011–2021). The mean (2.91 log copies/mL) was calculated from the first twenty viral load runs. The upper control limit (3.41 log copies/mL) and lower control limit (2.41 log copies/mL) were set as a 0.5 log difference from the mean. Four of the 35 kit lots used had a higher range shown in black oval. **b** Levey-Jennings chart of 321 viral load runs after removing the values of 4 lots (69 runs) showing a higher range. X axis depicts: number of runs. Y axis depicts: mean viral load in log copies/mL.

and quality assurance measures compromises the quality of laboratory results [14]. Our study shows that the performance of the LPC in 10 years, irrespective of the different batches of reagents used, was consistent and comparable with the IQC values, and the use of LPC over IQC has a lot of advantages. The LPC has non-infectious armoured RNA with HIV-1 sequences, and these armoured RNAs are relatively stable and have excellent lot-to-lot consistencies. Armoured RNA formulated with human plasma is stable at 4°C for at least 11 months and 37°C for at least 30 days [15]. As we see in the data provided in the graph, all the LPC values were within a 0.5 log difference from the mean value, irrespective of the lot variations. During our study, except for four lots, the LPC

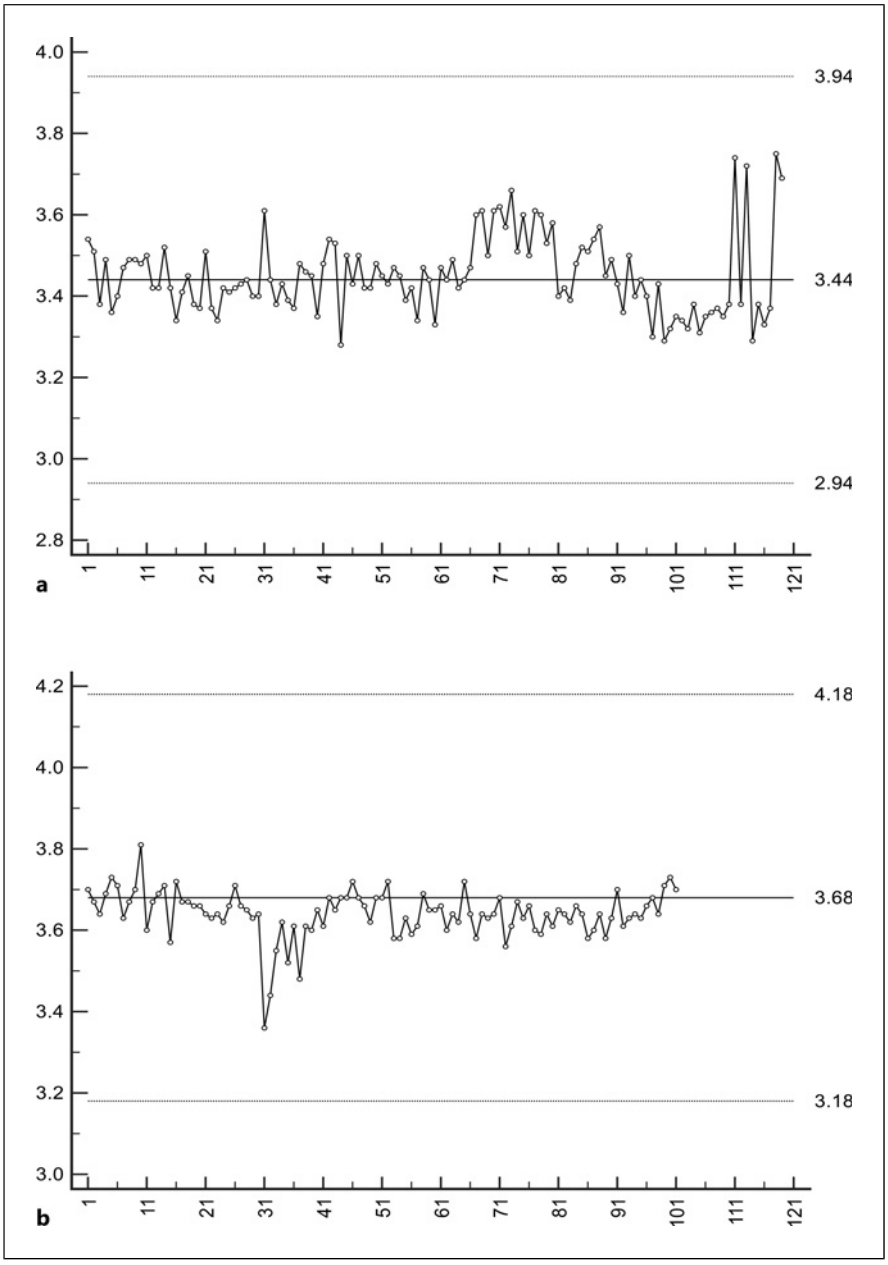

Fig. 2. Levey-Jennings control chart of two batches of HIV-1 IQC used along with 220 viral load runs. **a** First batch of IQC used for 119 runs using 13 different reagent lots. The mean was 3.44 log copies/mL, and the upper and lower control limits were set as ± 0.5 logs (3.94 and 2.94 log copies/mL), respectively. **b** The second batch of IQC was used for 101 runs using nine different reagent lots. The mean was 3.68 log copies/mL. The upper and lower control limits were set as ± 0.5 logs (4.18 and 3.18 log copies/mL). X axis depicts: number of runs. Y axis depicts: mean viral load in log copies/mL.

values of the other 35 lots were very close. Even in those four lots, the viral load level was higher but within 0.5 log. Therefore, our findings indicate that the use of a low positive control is a viable, cost-effective, and pragmatic option to monitor the performance of the assay in the long run, especially in resource-constrained settings. In conclusion, in HIV viral load laboratories where in-house quality control cannot be prepared, regular routine quality monitoring can be carried out by assessing the performance of the low positive control provided by the manufacturer.

Acknowledgment

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Statement of Ethics

This published work conforms with the guidelines for human studies and was conducted ethically in accordance with the Declaration of Helsinki. This study has been granted an exemption of ethical clearance (dated August 28, 2023) by the Institutional Review

Board (Ethics Committee), Christian Medical College, Vellore, India. A written informed consent was obtained from both the patients and blood donors for the use of their samples for the preparation of internal quality control, and this was approved by the Institutional Review Board (Ethics Committee), Christian Medical College, Vellore, India (IRB Minute No. 14461, dated February 23, 2022).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

John Paul Demosthenes: data curation, formal analysis, methodology, and writing – original draft. Ben Chirag Ghale: data curation and methodology. Diviya Alex: data curation, formal analysis, methodology, and writing – review and editing. Veena Vadhini Ramalingam: data curation, methodology, and writing – review and editing. Gnanadurai John Fletcher: formal analysis, investigation, project administration, resources, supervision, and writing – review and editing. Priya Abraham: project administration, resources, supervision, and writing – review and editing. Rajesh Kannangai: conceptualization, project administration, resources, supervision, validation, and review and editing. All authors have read and agreed to publish this version of the manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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