Comparative Performance of Cobas 6800, 8800 with Cobas Ampliprep/TaqMan for HIV Viral Load Quantification

Azuka Okwuraiwe, Joseph Shaibu, Aisha Gambari, Anthony Adeniyi, and Chika Onwuamah

ABSTRACT

Human immunodeficiency virus (HIV) remains a serious public health challenge, especially in Africa. Viral load (VL) is the major marker in monitoring disease prognosis. With the increased demand for HIV VL, countries are equipping fewer laboratories with higher throughput machines based on real-time polymerase chain reaction (PCR) technology. They are fully automated, faster, possess improved assay sensitivity/specificity, higher throughput, more dynamic ranges and reduced contamination rates. This study sought to verify the analytical performance of two new Cobas instruments against the Cobas Ampliprep/TaqMan (CAP/CTM), used in tracking viral suppression in Nigeria. In a cross-sectional study, aliquots of clinical HIV viral load samples were assayed on the three platforms. Values obtained with the CAP/CTM were compared with those from Cobas 6800/8800 in a routine clinical setting. Between June and August 2019, 100 plasma samples collected were analysed in parallel using both techniques. Accuracy, inter-assay precision, linearity and carry-over effect analyses were conducted. The correlation coefficients of 0.997, 0.874, and 0.878 were obtained for C8800/C6800; C8800/CAP/CTM, and C6800/CAP/CTM, respectively. The level of agreement using a Bland–Altman plot was 94.2%. The systems produced good analytical performance, comparable and correlated with CAP/CTM. Therefore, Cobas 6800 and 8800 machines are suitable and acceptable for clinical samples; and recommended for high-volume laboratories.

Keywords: HIV, PCR, quantification, viral load.

1. INTRODUCTION

The human immunodeficiency virus (HIV) is still a major public health challenge globally and particularly in Africa. Nigeria currently ranks fourth in the world with regard to HIV burden. Nigeria has a generalised HIV epidemic with the highest HIV burden in West and Central African sub-region [1]. The country has an estimated 1.8 million people living with HIV (PLHIV) [1]. Although there has been a 26% reduction in the number of AIDS-related deaths since 2010, the number of newly acquired HIV infections has increased in the same period [1]. Viral load is a major marker in disease prognosis and treatment of HIV infected patients as recommended by World Health Organization guidelines [2].

Along with clinical chemistry and immunological tests, HIV viral load (VL) is used to assess the efficacy of antiretroviral drugs. Therefore, accurate measurement of HIV VL is essential to provide clinicians with valuable information to determine treatment decisions. New quantitative HIV assays and innovative point of care (POC) devices (for smaller settings) have been designed to cope with the increasing molecular diversity of the virus and overcome the issue of turnaround time and the challenges of viral load estimation [3].

Laboratories that implement quality management systems (QMS) are required to verify any new technology or instrument that is introduced into the system, against the existing platform (a requirement of ISO 15189:2012) [4], [5]. Moreover, and more importantly, patients’ results that are generated have to be accurate, clinically feasible and interpretable by clinicians. Verification of new technology
also confirms manufacturers’ claims and provides assurance that the technology is of high-quality, precise and accurate; which is the rationale of this correlation study.

Roche Diagnostics (Mannheim, Germany) are the manufacturer of these precision instruments for clinical laboratory diagnostics. The striking difference between the fully automated platforms, the Cobas 6800 or Cobas 8800 and the Cobas Ampliprep/Cobas TaqMan (CAP/CTM) is throughput. CAP/CTM can process 24 samples (3 controls inclusive) in five and half hours, while the Cobas 6800 and Cobas 8800 can process 96 samples (3 controls inclusive) in 4 hours. This high throughput capability aligns perfectly with recent efforts of centralising laboratories in countries. Samples are transported in a cold chain from far remote distances to central or “mega” laboratories, in a hub and spoke model, which processes these samples and returns results in the shortest possible test-turnaround times (TAT).

HIV continues to be an epidemic in Africa, and HIV viral load is still the best biomarker to monitor disease progression and prognosis. In order to manage HIV, viral load determination has become more vital than ever.

In line with the United Nations target to end the HIV/AIDS epidemic by 2030, the 95-95-95 targets, ensuring that 95% of people living with HIV know their status, 95% of people who know their status are currently receiving treatment, and 95% of people on treatment have a suppressed viral load, [6] can only be achieved with large capacity instruments that can give accurate results in the shortest TAT. With increased demand for HIV VL, countries are now equipping fewer laboratories with higher throughput machines, aligning with the hub and spoke model [7]. These instruments based on real-time polymerase chain reaction (rtPCR) technology are faster, possess improved assay sensitivity/specificity, have higher throughput, larger dynamic ranges and reduced rates of contamination. The human contact with these mega machines while in operation is next to none [7].

In this study, we report the verification and comparative performance of the Cobas 6800 and Cobas 8800 analysers against the older CAP/CTM robust laboratory automated systems used in few tertiary health facilities in Nigeria.

2. Materials and Methods

2.1. Ethical Considerations

Ethical approval was obtained and the protocol and safety guidelines satisfied the conditions of NIMR Institutional Review Board (IRB) and policies regarding experiments that use specimens from human subjects.

2.2. Study Location

This comparative evaluation study was conducted at the Centre for Human Virology and Genomics (CHVG) of the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. CHVG is an ISO 15189:2012 accredited and WHO pre-qualification laboratory. The laboratory implements QMS and has endeavoured to offer premium service to its various clients. The study was carried out between June and August 2019.

2.3. Cobas Ampliprep/Cobas TaqMan HIV-1 Test, Version 2.0

This assay simultaneously targets the gag and the LTR region with two dually labelled hybridisation probes. The lower limit of quantitation is 1.30 log_{10} copies/ml, and the upper quantitation limit is 7.0 log_{10} copies/ml. It utilises 1000 μl of specimen volume. After samples are loaded in sample racks, nucleic acid extraction, amplification and detection are performed and controlled by the CAP/CTM Ampliclink v3.0 software. The CAP/CTM HIV-1 Test is based on three major processes: specimen preparation to isolate HIV1 RNA; reverse transcription of target RNA to form cDNA; and simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probes specific to the target.

2.4. Cobas 6800 and Cobas 8800 HIV-1 Quantitative Test

The cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800/5800/6800/8800 Systems targets two unique regions of the HIV-1 genome to improve genotype inclusivity, detect HIV-1 variants and potentially avoid under quantification. The rapidly mutating HIV-1 virus can evade quantification with a single-target, viral-load assay. The dual-target design combines with a new polymerase enzyme and an additional primer to increase mismatch tolerance further and expand inclusivity in non-B Group M viruses [8], [9].

2.5. Panels and Clinical Samples

Commercial clinical panels (Accurun Series, Sera Care Life Sciences, Milford, MA, 01757) were purchased for verification. Human plasma samples were also sourced from regular clinical patient viral load samples after voluntary informed consent was given.

Clinical plasma samples with high titers in the region of 9,000,000 RNA copies/ml (6 logs) were pooled, and a 10-fold dilution with human HIV-negative plasma was done, as shown in Table I.

2.6. Intra Assay Precision-Platform Comparison of 6800/8800 with CAP/CTM

A set of 30 samples were made into three aliquots of 1 ml each. One aliquot was assayed according to the manufacturer’s instructions on Cobas Ampliprep. The other two aliquots of the set of samples were assayed on the Cobas 6800 and 8800.

Comparison analyses were carried out on the existing CAP/CTM and new platforms, Cobas 6800 and Cobas 8800. Twenty-four sets of previously tested patients’ samples with six very high viral loads >10,000 cp/ml, six high viral loads >5,000 cp/ml, six low viral loads <1,000 cp/ml and six ≤20 cp/ml results for each equipment were tested in quadruplicate (x4) on CAP/CTM and Cobas 6800 and Cobas 8800.

The total number of sample tests on each piece of equipment was 96, making a total of 288 on the three pieces of equipment utilised.
TABLE I: HIV RNA Stock and Corresponding Serial Dilution Quantities Utilised for the Study

<table>
<thead>
<tr>
<th>Neat plasma Stock (RNA cp/ml)</th>
<th>9,000,000</th>
<th>8,000,000</th>
<th>6,000,000</th>
<th>4,000,000</th>
<th>2,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial dilution aliquots (RNA cp/ml)</td>
<td>900,000</td>
<td>800,000</td>
<td>600,000</td>
<td>400,000</td>
<td>200,000</td>
</tr>
<tr>
<td></td>
<td>90,000</td>
<td>80,000</td>
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<td>40,000</td>
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<td>90</td>
<td>80</td>
<td>60</td>
<td>40</td>
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2.7. Analytical Parameters

2.7.1. Accuracy

For analytical accuracy parameters, 45 samples with varied HIV viral titers were assayed on the Cobas 6800 and Cobas 8800 using 15 characterised samples (five negatives, five high positive and five low positive) at three concentrations. Each sample was tested three times within the same run, equivalent to 45 tests per equipment. Ninety samples were tested on the two equipment, excluding controls.

2.7.2. Inter-Assay Precision

For inter-assay precision, 15 high and 15 low viral load specimens were run in duplicate (n = 60 specimens). Specimens were tested one time on three different but consecutive days. The first test of the intra-assay precision served as the first day, followed by two additional consecutive assays of the same set of samples. Sixty samples were assayed on each piece of equipment per day for two days (60 × 2 × 2 = 240 tests).

2.8. Linearity (Analytical Measuring Range)

Linearity was determined by assaying 18 samples with serially increasing concentrations on the Cobas 6800 and Cobas 8800. The sample concentrations were spread through the assay’s dynamic range (43–2,220,000 RNA copies/ml).

2.9. Carry-Over Effect

In the test for carry-over effect, consecutive high and low-concentration samples were tested alongside each other to examine any instance of carrying over RNA from one test procedure or sample to another. Specimens with high and low titres were arranged intermittently and tested to ensure the system does not carry over contaminants from high-titre specimens to low-titre specimens. The same low-level control materials (5 aliquots) and high-level control materials (5 aliquots) were arranged intermittently in a predetermined order.

2.10. Statistical Analysis

Data curation was carried out using Microsoft Excel version 10. Means and standard deviations of values were obtained. Data were plotted on Bland Altman plots to determine the correlation and similarity between results from the Cobas 6800, 8800 and CAP/CTM.

3. Results

Bland Altman plots for Cobas 6800 compared to CAP/CTM, and Cobas 8800 compared to CAP/CTM are

Fig. 1. A Bland-Altman plot showing the degree of agreement between the Cobas 8800 and the CAP/CTM assays. The number of samples ranging within the confidence (mean ± 2SD) interval is 54 out of 60 (90%).

Fig. 2. A Bland-Altman plot showing the degree of agreement between the Cobas 6800 and the CAP/CTM assays. The number of samples ranging within the confidence (mean ± 2SD) interval is 54 out of 60 (90%).

Fig. 3. Correlation between the Cobas 6800 and Cobas 8800 HIV viral load results.
Fig. 4. Carry-over effect analysis of HIV VL on the Cobas 6800 and Cobas 8800 instruments.

shown in Figs. 1 and 2, respectively. They show that the mean HIV VL results of the two instruments are in close agreement. The correlation between the C6800 and C8800 can be seen in Fig. 3.

The correlation of 6800 and 8800 results with CAP/CTM is summarised below; the log values of CAP/CTM and Cobas 8800 were strongly and positively correlated (values are 0.96, 0.95, 0.96 and 0.97; p < 0.001).

In addition, the log values of CAP/CTM and Cobas 6800 were strongly and positively correlated (values are 0.96, 0.96, 0.97 and 0.95; p < 0.001), as shown in Fig. 3. There was no carry-over effect of samples on the both Cobas 6800 and 8800 instruments, as the sequential high and low viral load results can be seen in Fig. 4. Inter-assay coefficient of variation (CV) was calculated from the day 1 to day 3 viral load data, and we found it to be 15.8 and 7.1 for Cobas 8800 and Cobas 6800 respectively. The linearity of the instruments was conducted and the values ranged from 74 to 2,220,000 RNA copies/ml and 43 to 1,970,000 RNA copies/ml, on the Cobas 6800 and Cobas 8800, respectively (Figs. 5a and 5b). Viral load values were also accurate between the two as depicted in Fig. 6.

4. DISCUSSION

Whenever a new technology is deployed to a country health facility setting or laboratory, it should be verified for accuracy against the existing technology. This is good laboratory practice and is a standard procedure of a facility or laboratory that implements a quality management system. In this study, different characteristics were investigated, including the correlation, inter-assay precision, linearity, accuracy and carry-over effect.

In general, there was a reasonable correlation between the results of CAPCTM and the Cobas 6800 and Cobas 8800. No carry-over effect was observed during analysis, indicating that RNA from a preceding high-titre viral load sample would not be passed onto the following sample. This is vital and proves that inter-sample contamination is nil. Inter-assay precision or repeatability was also commendable (especially for unstable RNA molecules) and
the CV was low for the two instruments. The instruments have a defined linear range that indicates their measurements are close to reality. Previously, CHVG compared the tedious, time-consuming low throughput Roche Cobas Amplicor (manual specimen preparation/extraction and manual detection method) and the CAP/CTM [7]. In that study, the correlation coefficient for the two assays was 0.83, and the level of agreement was 94.2%. The analytical performance of Cobas 6800 for HIV-1, HBV and HCV assays was previously verified and evaluated against the CAP/CTM assays and found to correlate well [10], [11]. This present comparison study further demonstrates the reliability of ‘established players’ in development of robust molecular diagnostic systems. Doctors and patients need consistent accuracy of their results, which is the prime purpose of any laboratory, moreover, those that implement QMS.

The need for accurate diagnostics cannot be overemphasised in healthcare. The comparative performance of the Cobas 6800 and Cobas 8800 against the CAP/CTM was found to correlate closely. They can also assay other pathogens like hepatitis and human papilloma virus. Therefore, they are recommended machines for large tertiary facilities catering to a vast clientele.

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**CONFLICT OF INTEREST**

There are no competing interests from the authors.

**REFERENCES**


