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High correlation between the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1, v2.0 and the Abbott m2000 RealTime HIV-1 assays for quantification of viral load in HIV-1 B and non-B subtypes

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ABSTRACT

Background: HIV-1 viral load assays are critical tools to monitor antiretroviral therapy efficacy in HIV-infected patients. Two assays based on real-time PCR are available, the Abbott Real-Time HIV-1 assay (Abbott assay) and the new Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test, v. 2.0 (TaqMan® test v2.0).
Objectives: We have compared the performance of the two assays in 546 clinical plasma specimens of group M strains from Luxembourg and Rwanda.
Study design: Our analyses focused on subtype inclusivity and platforms accuracy for 328 low level viremia samples.
Results: Strong agreement and linear correlation were observed between the two assays ($R^2 = 0.95$) over a wide dynamic range. Bland–Altman analysis showed a mean difference of 0.04 log 10 indicating minimal overall viral load quantification differences between both platforms. One subtype C was severely under-quantified by TaqMan® test v2.0 for which sequence analysis revealed multiple mismatches between the viral sequence and the primer/probe regions. A non significant lower quantification of the Abbott assay was shown for subtype A1 with a mean log 10 difference of 0.24. For specimens under 200 cp/mL, the overall agreement was 90% at the cut-off of 50 cp/mL and 67% at assay's lower limit of detection of 20 and 40 cp/mL. 309 samples were retested by the COBAS® AMPLICOR® HIV-1 MONITOR Test, v. 1.5 and a lack of agreement between the three assays around their lower limit of quantification was revealed.
Conclusions: Both real-time tests were closely comparable in the quantification of viral load specimens of ten HIV-1 subtypes and recombinant forms.

1. Background

HIV-1 viral load (VL) assays are critical tools to monitor antiretroviral therapy (ART) efficacy in HIV-infected patients. VL is the major prognostic marker to predict disease progression and for timing initiation of ART.[1,2] The COBAS® AMPLICOR® HIV-1 MONITOR Test v1.5 (HIM v1.5) based on end-point amplification technique has been widely used in clinical practice and a viral load of 50 cp/mL has been defined as a medical decision point for clinical failure.[4] Real-time technologies with improved sensitivity and broader dynamic range has now reached the market. The Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test has indeed increased the frequency of detectable plasma HIV-1 viral load and blip rate.[5–7] The clinical relevance of low level viremia defined as viremia detected on specimens with HIM v1.5 test results <50 cp/mL is still under investigation. However failure to correctly quantify non-B subtype viruses has been observed, a finding which is of concern at the time when increasing prevalence of these subtypes is being reported.[8,9] Moreover, several African national

Abbreviations: HIV, Human Immunodeficiency Virus; AIDS, Acquired Immune Deficiency Syndrome; RT-PCR, Reverse-Transcriptase Polymerase Chain Reaction; RNA, Ribo Nucleic Acid; LOD, Lower Limit of Detection; VL, viral load; cp/mL, copies number/mL; ART, antiretroviral therapy; Abbott assay, Abbott Real-Time HIV-1 assay; TaqMan® test v2.0, Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test v. 2.0; HIM v1.5, COBAS® AMPLICOR® HIV-1 MONITOR Test v1.5 HIM v1.5; CI, confidence interval.

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programs recommend VL monitoring twice a year and automated real-time assays need to provide an accurate quantification of the non-B variants predominant in these regions.[10,11] Therefore, the worldwide high genetic diversity of HIV calls for highly efficient real-time assays tolerant to the natural polymorphisms present in the primers/probe sequences that may lead to underestimation of HIV-1 RNA levels.

Two highly sensitive assays based on real-time PCR technology are now available, the Abbott m2000 Real-Time HIV-1 assay (Abbott assay) and the new Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test, v. 2.0 (TaqMan® test v2.0) with a detection limit of 40 cp/mL and 20 cp/mL, respectively.[12–14] The TaqMan® test v2.0 has been specifically developed to improve the subtype inclusivity of its predecessor version.

2. Objectives

We have compared the performance of the Abbott assay and the TaqMan® test v2.0 in 546 clinical plasma specimens of group M strains. Our analyses further focused on accuracy of both platforms for 328 low level viremia samples and 309 samples were reanalyzed using the HIM v1.5a platform.

3. Study design

3.1. Clinical sample collection

Whole EDTA blood samples were collected from January to November 2009 from HIV-infected treatment-naïve-and ART-treated patients. The study has been performed in Luxembourg with anonymized samples from routine leftover (Centre Hospitalier de Luxembourg) and in Rwanda with anonymized samples from the National ART program (Reference National Laboratory, Kigali), each Rwandese patient have signed a written informed consent form for the study. After centrifugation, plasma samples were aliquoted and stored at −80 °C before analysis in each collecting site. Rwandese plasma samples were shipped on dry ice to Luxembourg. For subtype determination, sequencing of RT and PRO genes was performed using Viroseq v2.0 technology (Abbott, Germany) on an ABIprism 3130 xl (Applied Biosystems, Belgium). Subtypes were assigned using the REGA and COMET HIV-1 subtyping tools against Genbank reference HIV-1 group M sequences. For samples below 1.000 cp/mL, subtype was assigned on a previous plasma sample of the patient > 1.000 cp/mL. In total, 168 B and 378 non-B samples were selected: 102 A1, 79 C, 22 CRF01-AE, 66 CFR02-AG, 14 CRF06-CPX, 1D, 24 F1, 3 F2 and 67 G from patients with or without ART.

3.2. Viral load assays

VL quantification was first performed using the Abbott assay following manufacturer's instructions with 0.6 mL of plasma in Luxembourg. The Real-Time Abbott assay uses partially double-stranded probes with non competitive fluorescent detection[12,13] binding to the HIV-1 pol INT gene. The 546 selected samples were retested with the Roche TaqMan® test v2.0 in Luxembourg using 0.85 mL plasma of a separate aliquot without intermediate freeze thawing according to the manufacturer's protocol within three months after the first VL determination. TaqMan® test v2.0 uses TaqMan probes with fluorofore and quencher (5′ nuclease probe breakage) binding the gag and ltr genes. 309 available samples of low viraemic load (<200 cp/mL) were reanalyzed on a separate aliquot using the HIM v1.5 at the University Hospital Regensburg, Germany.

3.3. Sequence analysis of the discordant subtype C virus sample

One subtype C specimen exhibiting a severe underquantification by TaqMan® test v2.0 was sequenced in the assay target regions at Roche Molecular Systems, Pleasanton, USA. HIV-1 sequences flanking the gag and ltr regions were amplified. Amplicons were sequenced on an ABIprism 3130 xl using the BigDye terminator cycle-sequencing chemistry (Applied Biosystems). The obtained sequence information was compared to HIV-1 sequences from the Roche Diagnostics Global Surveillance HIV-1 sequences dataset to identify polymorphisms affecting the performance of the assay.

3.4. Statistical analysis

All assays results were reported in copy numbers/mL (cp/mL) and transformed into $\log_{10}$ cp/mL for statistical analyses. An absolute difference of more than 0.9 $\log_{10}$ was defined as severely discrepant, between 0.5 and 0.9 $\log_{10}$ cp/mL was moderately discrepant and below to 0.5 $\log_{10}$ cp/mL was non-discrepant. Agreement and correlation between the two assays was evaluated using Bland–Altman analysis and Deming regression analysis respectively. Percent agreement on low vireamic samples was evaluated based on 3-dimensional contingency tables, corresponding confidence intervals were calculated using the Wilsons efficient-score method. Statistical analysis was done using Microsoft Excel and Software WinMC.

4. Results

4.1. Agreement and correlation between the two viral load assays

543 of 546 clinical specimens could be used for platform comparison as 3 samples failed in the TaqMan® test v2.0 assay. Bland–Altman analysis of all HIV-1 RNA positive samples ($n = 289$) with quantifiable titers within the tests' dynamic range revealed a high agreement between the two real-time assays (Fig. 1A) demonstrated by a mean difference of 0.04 $\log_{10}$ cp/mL and a 95% confidence interval (95% CI) of 0.01–0.08. 20 samples showed slightly discrepant results, among them 12 subtype A1 samples displaying a higher quantification with the TaqMan® test v2.0 assay. After Deming regression analysis, a high correlation was observed between TaqMan® test v2.0 and the Abbott assay (Fig. 1B) over a wide dynamic range. One subtype C displayed a severe under quantification by more than 1 $\log_{10}$ in the TaqMan® test v2.0 as compared to the Abbott assay and the result of the Abbott assay was confirmed by the HIM v1.5 assay (data not shown). After sequence analysis, several mismatches were observed in both the gag and LTR assay target regions which may explain the underquantitation of this sample by TaqMan® test v2.0 (data not shown).

4.2. Subtype inclusivity

Bland–Altman analyses for each main non-B subtype of our sample collection were performed (Fig. 2). A lower quantification of the Abbott assay compared to the TaqMan® test v2.0 was shown for subtype A1 samples with a mean log difference of 0.24 (95% CI: 0.18–0.31). Noteworthy, all A1 discrepant samples were collected in Rwanda and displayed a VL predominantly under 4 $\log_{10}$ cp/mL. Seven moderately discrepant samples could be re-analyzed with HIM v1.5 and confirmed titer results similar to those obtained with TaqMan® test v2.0 (data not shown).

4.3. Low viremic samples

328 samples with a VL below 200 cp/mL were compared, these included also samples with unquantifiable titer below the tests'