Evaluation of the new Aptima® HIV-1 Quant Dx assay for HIV-1 RNA quantification in plasma of infected individuals: a comparison with Abbott RealTime HIV-1 assay

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Introduction

The Aptima® HIV-1 Quant Dx assay (Hologic, Inc.) is a new method for detection and quantification of HIV-1 viral RNA in plasma from HIV-1 infected individuals. It is based on real-time Transcription-Mediated Amplification technology targeting two regions (pol and 5'LTR) of the HIV-1 genome. The assay was developed for use on the automated Panther® platform.

Aim

This study compared the performance of the Aptima® HIV-1 Quant Dx assay to the FDA approved Abbott RealTime HIV-1 assay in testing analytical standards and clinical samples.

The low limit of detection of the Aptima® HIV-1 Quant Dx assay (Hologic, Inc.) is 13.1 copies/ml; the linear quantification range spans from 30 copies/ml to 10,000,000 copies/ml.

For the Abbott RealTime HIV-1 procedure, the low limit of detection is 40 copies/ml and the linear range of quantification is 40 copies/ml to 10,000,000 copies/ml. The Abbott assay was performed on the m2000sp/rt instruments.

Reagents and Methods

The evaluation was performed using:

1. 220 clinical plasma samples (with viral load range spanning from not-detected HIV-1 RNA to >1x10^6 cp/ml HIV-1 RNA),
2. the WHO 3rd HIV-1 International Standard (WHO-IS; NIBSC code: 10/152),
3. the WHO 2nd International Reference Panel Preparation for HIV-1 Subtypes for NAT (WHO-ST; NIBSC code: 12/224) and
4. the QCMD HIV-1 RNA EQA panel (QCMD).

Residual plasma samples were obtained from HIV-1 infected individuals attending the out-patient care facility of the "L.Spallanzani" Hospital in Rome for routine monitoring of HIV-1 viremia. Specimens were stored at –80° C and analysed with both assays in the same working day.

Viral load assays were used according to manufacturers' instructions.

All viral load data were analysed as log$_{10}$-transformed values. In the correlation analysis, the low limit of detection of both assays was lined up to 1.60 log$_{10}$ copies/ml. The concordance of qualitative results between the Aptima assay and the reference test was established by Cohen's kappa statistic. The correlation between the quantitative results was evaluated as the concordance correlation coefficient (ccc) of the measurements, according to Lin (1). The agreement between the assays was assessed with the Bland-Altman plot (2). Accuracy and reproducibility of quantification was evaluated using the WHO-IS. The ability to measure HIV-1 subtypes was assessed on the WHO-ST and QCMD panels.

Results 1/3

With **clinical samples**, concordance between two assays for qualitative results was high (91.8%) with a Cohen's kappa statistic equal to 0.836. In comparison of samples with quantitative results >40 copies/ml in both assays (n=93), concordance was very high, with a Lin's ccc interassay concordance of 0.980 (p<0.0001) (**Fig.1**). Mean differences of measurement between assays, according to Bland-Altman method, was low (0.115 log$_{10}$ cp/ml), with Aptima assay giving slightly higher results at high levels of viremia (**Fig.2**).
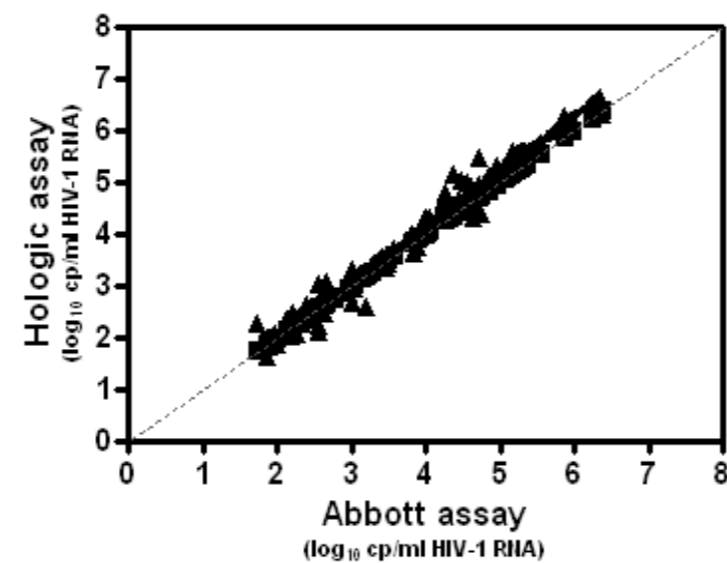

Fig.1: Log$_{10}$-transformed viral load results of 93 clinical samples given as >1.60 log$_{10}$ copies/ml (>40 copies/ml) with both assays. Concordance graph of the observed data, with linear regression line (solid) and line of perfect concordance (dashed).
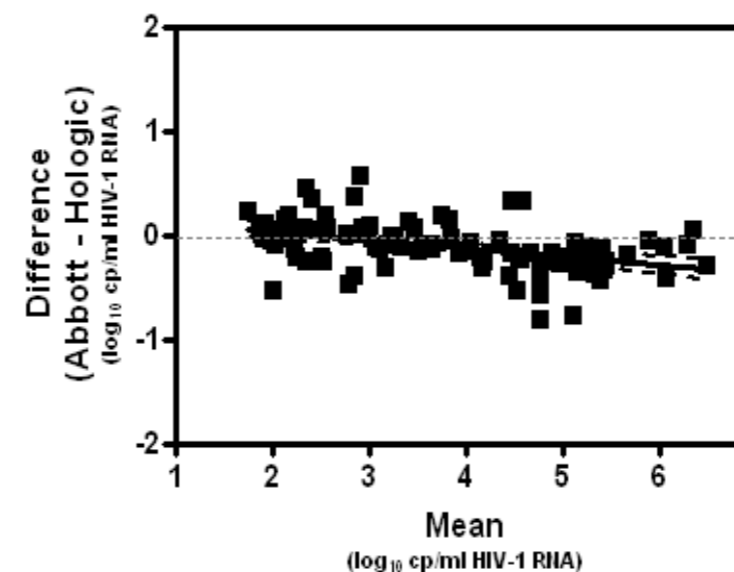

Fig.2: Bland-Altman plot of viral load values of 93 clinical samples given as >1.60 log$_{10}$ copies/ml (>40 copies/ml) with both assays, with linear regression line (solid) and line of perfect concordance (dashed).

Results 2/3

The **WHO 3rd HIV-1 International Standard** (diluted from 2000 copies/ml to 31 copies/ml) was quantified by Aptima assay at expected values, with excellent linearity (R^2 >0.970) within this range (**Fig. 3**). Reproducibility was very high, even at HIV RNA values in the lower part of the dynamic range. In **Fig. 4**, WHO 3rd HIV-1 International Standard results obtained with Aptima and Abbott assays by comparison.
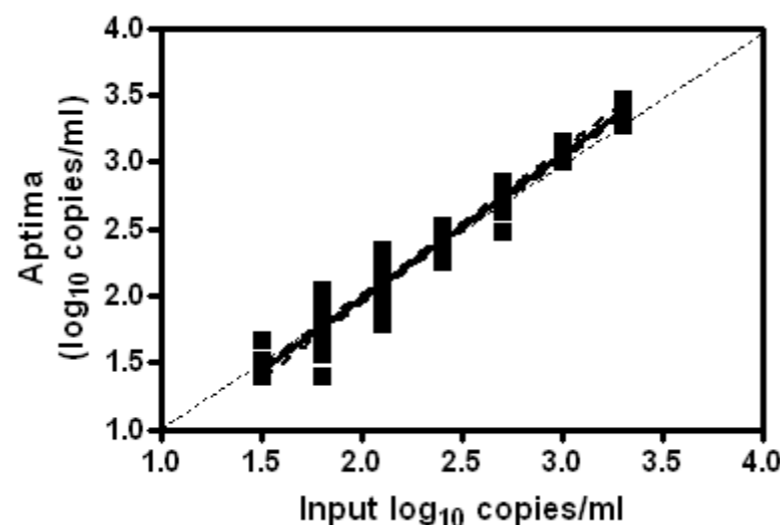

Fig.3: Log$_{10}$-transformed viral load results of the WHO 3rd HIV-1 International Standard (diluted at 2000, 1000, 500, 250, 125, 62.5, 31 copies/ml). Concordance graph of the observed data with Aptima assay versus expected values, with linear regression line (solid) and line of perfect concordance (dashed).
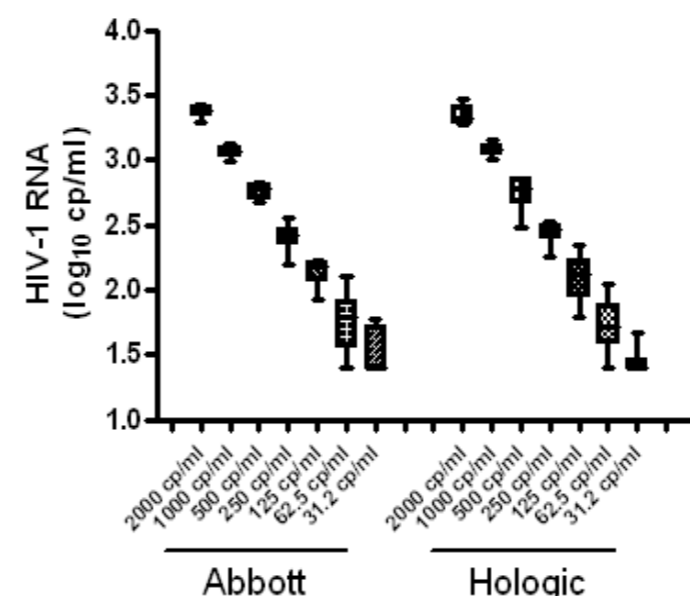

Fig. 4: Log$_{10}$-transformed viral load results of the WHO 3rd HIV-1 International Standard obtained with Abbott and Aptima assays. Median, quartiles, and extremes of the data are shown for each dilution.

Results 3/3

The Aptima assay, as Abbott procedure, was able to accurately quantify all the main **HIV-1 subtypes** in both the WHO 2nd International Reference Panel Preparation for HIV-1 Subtypes for NAT (**Fig. 5**) and QCMD HIV-1 RNA EQA reference panel. (**Fig.6**).
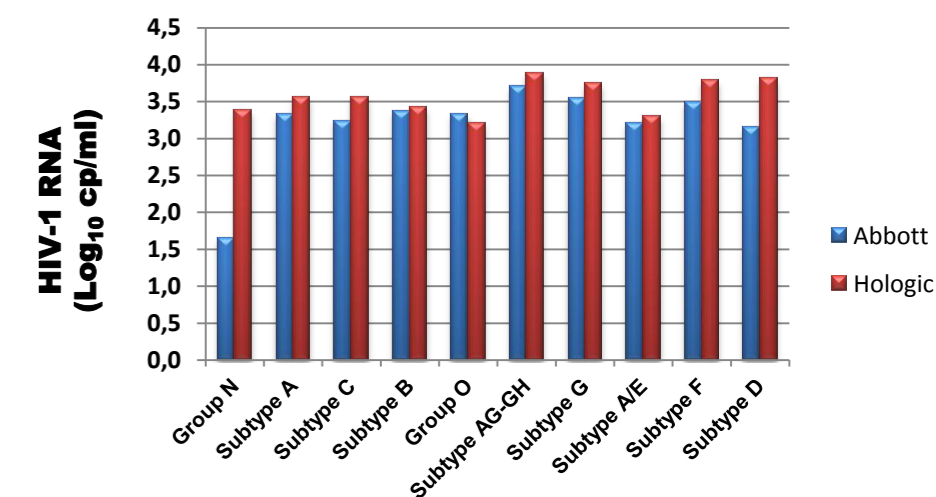

Fig.5: Log$_{10}$-transformed viral load results of the WHO 2nd International Reference Panel Preparation obtained with Hologic Aptima (red) and Abbott (blue) assays.
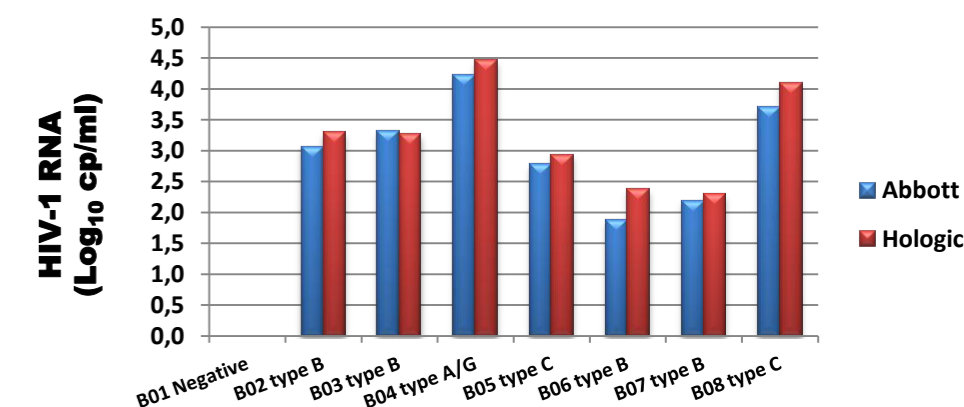

Fig.6: Log$_{10}$-transformed viral load results of the QCMD HIV-1 RNA EQA reference panel obtained with Hologic Aptima (red) and Abbott (blue) assays.

Conclusion

The recently CE-IVD approved Aptima® HIV-1 Quant Dx assay shows high performance characteristics that can be considered equivalent to those of the reference diagnostic system (Abbott RealTime HIV-1).

Along with excellent performance, the automation and improved workflow of the Aptima assay on the Panther system make it a good choice for routine monitoring of HIV-1 viral load.

A remarkable characteristic of the new assay is the elevated accuracy and reproducibility, even at low HIV RNA values.

References:
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