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# Evaluation of the Hologic (Gen-Probe) Aptima HIV-1 RT Quantitative Assay on HIV-1 Subtypes


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## BACKGROUND

The Hologic Aptima HIV-1 Quant Dx Test, currently under development for use on the automated Panther® platform, is designed to detect and quantify HIV-1 viral RNA in plasma from HIV-1 infected individuals. The assay is intended for use in clinical monitoring of virologic response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. The highly, complex genetic diversity of HIV-1 virus presents a challenge to developers of HIV-1 nucleic acid tests, as each manufacturer targets different regions of the viral genome and uses a variety of multiplex approaches to allow detection of all subtypes. The performance of the Aptima HIV-1 Quant Dx was evaluated using a comprehensive panel of cultured virus and plasma samples collected worldwide and compared to that of two FDA cleared assay platforms: the Roche COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 v2.0 and the Abbott m2000 RealTime HIV-1 Tests.

## METHODS

**Study Design:** The performance of the Hologic Aptima HIV-1 Quant Dx on the Panther System was assessed for precision, accuracy, linear dynamic range and Lower Limit of Detection (LLOD). The ability of the assay to accurately quantitate HIV-1 subtypes was evaluated on 171 well-characterized HIV-1 cultured virus obtained from Walter Reed Army Institute of Research (WRAIR) (Brown et al, 2005), EQAPOL (Manak et al, 2012), NED (Huang et al,. 2002) and four Group O isolates (SeraCare, Inc. Gaithersburg, MD), representing 33 countries worldwide. Subtype designations were assigned based on full genome sequence analysis. Additional evaluation of subtype performance was conducted on 105 plasma specimens collected under Institutional Review Board and Ethics committee approvals in clinical studies located at US Military HIV Research Program (MHRP) sites in Thailand, Uganda, Tanzania, Kenya and Nigeria. Subtype designations for plasma samples were assigned based on Multiregion Hybridization Assay (MHA) analysis. The HIV-1 subtypes tested in this study included A, B, C, D, F, G, H, CRF04_cpx, CRF01_AE, CRF02_AG and various recombinants.

**Reagents and Methods**: Well characterized panels of HIV-1 subtypes were prepared by spiking of HIV-1 cultured virus into HIV negative EDTA plasma (Biospecialty Laboratory, Comar, PA) or Basematrix (SeraCare, Inc. Gaithersburg, MD). Lower limit of detection (LLOD) and quantification (LLOQ) were determined by testing thirty replicates each of 2-fold serial dilutions of cultured HIV-1 subtype B virus from 1.5 to 100 copies/ml. Subtype quantitation was evaluated on 171 HIV-1 isolates diluted to ~1E5 copies/ml. Linearity was also assessed using one selected viral isolate for each of the HIV-1 subtypes A, B, C, D, CRF01_AE and CRF02_AG at dilutions ranging from 1E2 to 1E6 copies/ml. Plasma specimens were diluted in Basematrix to approximately 1E4 copies/ml for testing, and the results of the Hologic Aptima assay were compared to those obtained by the Roche COBAS® TaqMan® HIV-1 v2.0 and the Abbott RealTime HIV-1 tests.

## RESULTS

**Precision and Linearity**: All Aptima calibrators performed at expected target values and exhibited excellent linearity between 10 and 10,000,000 copies/ml ($R^2$ >0.998). The precision of the Aptima HIV-1 Quant Dx between runs ranged from 2.8-10.4% at the 100 copy/ml level (mean 6.67%), and 0.22-0.47% for the 10,000,000 copy/ml level (mean 0.37%). The kit calibrator was run in triplicate over 11 runs and averaged 2.66 +/- 0.05 log copies. The kit controls over 11 runs averaged 2.71 +/- 0.13 and 4.99 +/-0.04 log copies/ml for the low and high control respectively.

**Accuracy and Linear Dynamic Range**: The AcroMetrix® HIV-1 Quantitation Panel (Life Technologies. Inc.) was used to assess the accuracy and linear dynamic range of the Aptima HIV-1 Quant Dx Test. Excellent linearity was observed from 100 to 5,000,000 copies/ml with $R^2$ values of 0.998 or higher. All results were within 0.15 log of the target value, with the exception of the 100 copies/ml sample tested on Day 1 which was within 0.32 logs. Linear dynamic range of 100 to 1,000,000 copies/ml was also demonstrated for six HIV-1 subtypes (A, B, C, D, CRF01_AE and CRF02_AG) selected from the HIV-1 WRAIR panel, with $R^2$ values >0.992 as is shown in Figure 1. Target values were assigned based on Abbott HIV-1 results used in preparation of the panel.

**Lower Limit of Detection (LLOD):** The LLOD of the Aptima HIV-1 Quant Dx Test was calculated based on the MN subtype B isolate at 7 concentrations with 30 replicates tested at each concentration. Probit Analysis of the data calculates the LLOD at 3.1 copies/ml at the 50% level and 15 copies/mL at the 95% level (Figure 2).

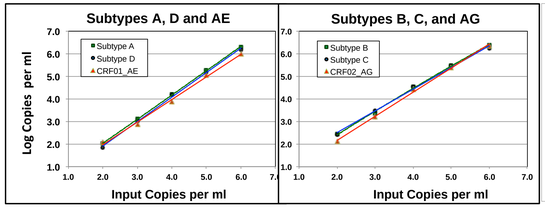



**Figure 1. Linearity and Subtype Performance of the Hologic Aptima HIV-1 RNA Quant Dx Assay.**

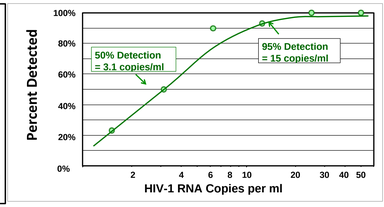



**Figure 2. Lower Limit of Detection (LLOD).**

| | TOTAL | United States | Venezuela | Uruguay | Brazil | Bolivia | Romania | Poland | Germany | Spain | France | Greece | Algeria | South Africa | Angola | Zaire | Cameroon | Tanzania | Zambia | Djibouti | Ethiopia | Ghana | Kenya | Malawi | Nigeria | Rwanda | Senegal | Somalia | Uganda | Kazakhstan | Thailand | India | China | Indonesia | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | US | VE | UY | BR | BO | RM | PL | DE | ES | FR | GR | DZ | SA | AN | CD | CM | TZ | ZM | DJ | ET | GH | KE | MW | NG | RW | SE | SO | UG | KZ | TH | IN | CN | ID | |
| B | 36 | 19 | 1 | 1 | 3 | 1 | | | 3 | 1 | 1 | 3 | | | | | | | | | | | | | | | | | | 3 | | 1 | | | |
| C | 37 | 1 | | | 1 | | | | | 1 | | | | 14 | 1 | | | 3 | 1 | 1 | 3 | | 1 | 1 | 1 | | 2 | 2 | 2 | | | 1 | | | 1 |
| CRF01_AE | 39 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 34 | | 3 | 2 | |
| CRF02_AG | 33 | 1 | | | | | | | 1 | | | | | | 1 | | 12 | | | 3 | | | | | 15 | | | | | | | | | | |
| A | 31 | | | | | | | | | 1 | | | | | | | | 2 | | | | | 1 | 8 | | 1 | 1 | | 16 | 1 | | | | | |
| D | 24 | | | | | | | | 2 | | | | | | | | 1 | | | | | | 1 | | | | | 1 | 19 | | | | | | |
| ACD, CD, AD, AC | 17 | | | | | | | | | | | | | | | | | 4 | | | | | 1 | | | | | | 12 | | | | | | |
| CRF01/B | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | | | | | |
| G/CRF02 recombinants | 14 | | | | | | | | | 1 | | | | | | | | | | | | | | | 13 | | | | | | | | | | |
| G | 20 | | | | | | | | | 2 | | | | | | 1 | 1 | | | | | | 1 | | 15 | | | | | | | | | | |
| F | 7 | | | | 3 | | 1 | | | 1 | | | | | | | 2 | | | | | | | | | | | | | | | | | | |
| H | 1 | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | |
| GROUP O | 4 | 1 | | | | | | | | 1 | | | | | | | 2 | | | | | | | | | | | | | | | | | | |
| CRF04_cpx | 4 | | | | | | | | | | | 4 | | | | | | | | | | | | | | | | | | | | | | | |
| CRF06_cpx | 1 | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | |
| CRF11_cpx | 1 | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | |
| CRF14_BG | 1 | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A1B | 1 | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| BF | 2 | | | | | | | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL | 277 | 22 | 1 | 1 | 8 | 1 | 1 | 3 | 4 | 11 | 3 | 4 | 1 | 14 | 2 | 2 | 18 | 9 | 1 | 4 | 3 | 1 | 12 | 1 | 46 | 1 | 3 | 2 | 49 | 1 | 40 | 1 | 4 | 2 | 1 |

**Table 1. Geographic Distribution of HIV-1 Subtypes Tested.**

*The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.*

## RESULTS

**Subtype Specificity:** A total of 171 HIV-1 culture isolates and 105 clinical specimens from 33 countries representing all the major Group M and Group O HIV-1 subtypes were tested (Table 1). The Aptima HIV-1 Quant Dx test quantified on the average 0.21 +/- 0.25 and 0.31 +/- 0.24 logs higher than the Roche TaqMan and Abbott RealTime tests, respectively. In comparison with the Abbott test, 18% (50/276) of the samples were quantified at greater than 0.5 log copies/ml, with the largest variability in subtypes C (49%, 18/37), F (43%, 3/7) and CRF02 Rec (36%, 5/14). Two clinical specimens from Nigeria showed >1.0 log copies/ml difference in the Abbott RealTime Test: a CRF02_AG at -1.09 and a G at -1.68. For the Roche test, 12% (34/276) were greater than 0.5 log copies/ml, with subtypes G (50%, 10/20) and CRF02 Rec (43%, 6/14) having the most variability (Figure 3).

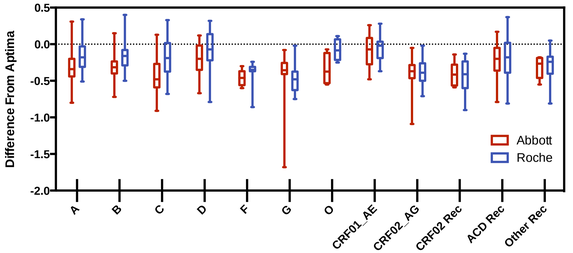



**Figure 3: Box-and-Whisker Plot of the Log Differences in HIV-1 Viral RNA Quantitation with respect to the Hologic Aptima HIV-1 Quant DX Assay.** Log differences of the HIV-1 RNA concentrations between the Hologic Aptima and Roche (blue) or Abbott (red) are plotted for all cultured virus and clinical specimens. The median, quartiles, and extremes of the data are shown for each subtype, with various recombinants grouped by category. Rec = recombinants.

## CONCLUSIONS

The Hologic Aptima HIV-1 Quant Dx Test performed on the automated Panther® System demonstrated excellent precision, sensitivity and linear dynamic range.

The Hologic Aptima HIV-1 Quant Dx having 50% detection of 3.1 RNA copies/ml, appears to have better sensitivity than the Aptima Qualitative HIV-1, Roche TaqMan® v2.0 or the Abbott RealTime tests.

The assay was capable of accurate quantification of all major HIV-1 subtypes in Group M to include A, B, C, D, G, H, F, CRF01_AE, CRF02_AG and other complex recombinants. Four HIV-1 Group O viral isolates were also quantified well by all three tests.

The Hologic Aptima HIV-1 Quant Dx test results correlated well with the HIV-1 RNA results obtained in the Roche TaqMan v2.0 and Abbott RealTime tests.

The fully automated assay is easy to use with up to 120 samples tested in under four hours. The use of lyophilized reagents improves reagent stability which is useful when shipping to thermo-challenging settings.


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