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 it monitors changes in plasma RNA levels. The highly complex genetic diversity of HIV-1 virus presents a challenge to developers of HIV virus nucleic acid tests, as each manufacturer specifies different regions of the viral genome and uses a variety of multiplex approaches to allow detection of all three subtypes.

To determine precision and linearity, between 10 and 10,000,000 copies/ml (R² >0.998). The precision of the Aptima HIV-1 Quant Dx between runs ranged from 2.17 +/- 0.13 to 4.98 +/- 0.49 log copies/ml for the low and high control, respectively.

HIV-1 culture isolates (171) from 33 countries and 105 clinical specimens were used to determine accuracy and linear dynamic range. The accuracy of the Aptima HIV-1 Quant Dx Test performed on the automated Panther® System was evaluated using a comprehensive panel of cultured virus and plasma samples collected worldwide and compared to that of two FDA cleared assays platforms, the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Quantitative Assay and the Abbott m2000 RealTime HIV-1 Tests.

Subtype Specificity:

Subtype B, C, and AG

Subtypes B, C, and AG

RESULTS

Precision and Linearity: All Aptima calibrators performed at expected target values and exhibited excellent linearity between 10 and 10,000,000 copies/ml. The precision of the Aptima HIV-1 Quant Dx between runs ranged from 2.8-10.4% at the 100 copies/ml level (mean 6.67%), and 0.22-0.47% for the 10,000,000 copies/ml level (mean 0.37%). The kit calibrators run in triplicate at 1.1 runs and averaged 2.86 +/- 0.05 log copies/ml. The kit controls ran in 11 runs averaged 2.71 +/- 0.15 and 4.94 +/- 0.49 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 10 to 10,000,000 copies/ml with R² 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 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 Hologic WRAIR panel, with R² values >0.992 as is shown in Figure 1. Target values were assigned based on Abbott m2000 results used in preparation of the panel.

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 (LLD). The ability of the assay to accurately quantitate HIV-1 subtype 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.) representing 33 countries worldwide. Subtype characterization was based on unique sequence analysis. Additional evaluation of subtype performance was conducted on 105 plasma specimens collected under Institutional Review Board and Ethics committee approval in clinical studies located at US Military HIV Research Program (MHRRP) sites in Thailand, Uganda, Tanzania, Kenya and Nigeria. Subtype assignments for plasma samples were based on Multigene Hydration Assay (MHA) analysis. The HIV-1 subtype(s) tested in this study included A, B, C, D, F, G, CRF01_AE, CRF02_AG, CRF03_Cpx, and CRF19_Ba/CRF25_Ba.

Reagents and Methods: Well characterized panels of HIV-1 subtypes were prepared by spiking of HIV-1 cultured viruses in HIV negative EDTA plasma (Respiratory Laboratory, Camar, PA) or Bassetomix (SandCares, Inc. Gathirdsworld, MD). Lower limit of detection (LLD) and quantification (LCQ) were determined by testing twenty replicates each of 2-fold serial dilutions of cultured HIV-1 subtype B virus from 1 to 100 copies/ml. Subtype quantification 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 1E5 copies/ml. Plasma specimens were added to Hank’s balanced salt solution to a hematocrit of approximately 16 E6 copies/ml for testing, and the results of the Hologic Aptima assay were compared to those obtained by the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 v2.0 and the Abbott m2000 RealTime HIV-1 tests.

RESULTS

Subtype Specificity: HIV-1 culture isolates (171) from 33 countries and 105 clinical specimens were used to determine accuracy and linear dynamic range. The performance of the Aptima HIV-1 Quant Dx Test, among the three assays were comparable, with the Roche and Abbott assays running about 0.3 to 0.6 lower than the Hologic Aptima. Two clinical specimens from Nigeria showed more than a log difference in RNA value in the Abbott RealTime Test: a CRF02_AG isolate from Nigeria showed more than a log difference in RNA value in the Abbott RealTime Test: a CRF02_AG isolate

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

Figure 2. Lower Limit of Detection (LLD) of the Hologic Aptima HIV-1 Quant Dx Test.

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 90% 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 HIV-1 tests.

The assay was capable of accurate quantification of all major HIV-1 subtypes in Group M to include A, B, C, D, F, G, CRF01_AE, CRF02_AG, CRF03_Cpx, and CRF19_Ba/CRF25_Ba.

Four HIV-1 Group O viral isolates were also quantified well in all three tests. The Hologic Aptima HIV-1 quantified on average 0.21 +/- 0.25 and 0.31 +/- 0.24 log copies/ml higher than the Roche TaqMan® and the Abbott RealTime HIV-1 respectively.

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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References:


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