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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).*

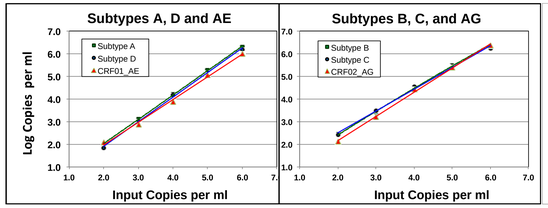

Figure 1. Linearity and Subtype Performance of the Hologic Aptima HIV-1 RNA Quant Dx Assay.

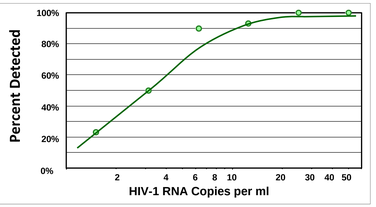

Figure 2. Lower Limit of Detection (LLOD).

	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: *HIV-1 culture isolates (171) from 33 countries and 105 clinical specimens from five countries, representing all the major Group M HIV-1 subtypes and HIV-1 Group O were tested on all three assay platforms. The specific subtypes and Country of Origin are summarized in Table 1. HIV-1 RNA values obtained 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 at -1.09 and one G at -1.68 (Figure 3). The greatest differences in RNA concentrations among the three tests were observed in clinical specimens from Nigeria (CRF02_AG, G and recombinant G/AG).*

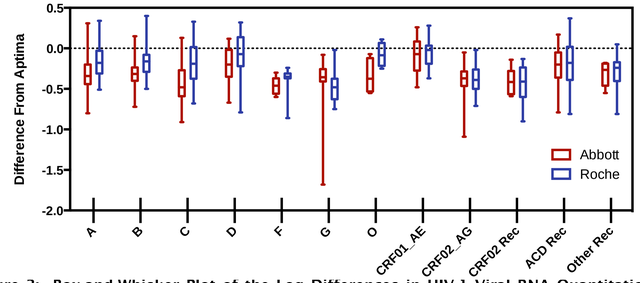

Figure 3: Box-and-Whisker Plot of the Log Differences in HIV-1 Viral RNA Quantitation with respect to the Hologic Aptima HIV-1 Quant DX Assay. *Log differences of the HIV-1 RNA concentrations between the Hologic Aptima and Roche (blue) or Abbott (red) are plotted for all cultured virus and clinical specimens. 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 quantified on average 0.21 +/- 0.25 and 0.31 +/- 0.24 log copies/ml higher than the Roche TaqMan v2.0 and 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:

Brown B.K. et al. J. Viol. 79: 6089-6101, 2005.
Huang D, et al. AIDS Research and Human Retroviruses. 19(4): 321-328, 2003.
Manak M. et al. AIDS Hum Retro Res, 28 (6); 594-606 2012.