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Performance of the Aptima® HIV-1 Quant Dx Assay on the Panther System

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***Abstract*—**The Aptima® HIV-1 Quant Dx Assay is a fully automated assay on the Panther system. It is based on Transcription-Mediated Amplification and real time detection technologies. This assay is intended for monitoring HIV-1 viral load in plasma specimens and for the detection of HIV-1 in plasma and serum specimens.

Nine-hundred and seventy nine specimens selected at random from routine testing at St Thomas' Hospital, London were anonymised and used to compare the performance of the Aptima HIV-1 Quant Dx assay and Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. Two-hundred and thirty four specimens gave quantitative HIV-1 viral load results in both assays. The quantitative results reported by the Aptima Assay were comparable to those reported by the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 with a linear regression slope of 1.04 and an intercept on -0.097.

The Aptima assay detected HIV-1 in more samples than the COBAS assay. This was not due to lack of specificity of the Aptima assay because this assay gave 99.83% specificity on testing plasma specimens from 600 HIV-1 negative individuals. To understand the reason for this higher detection rate a side-by-side comparison of low level panels made from the HIV-1 3rd international standard (NIBSC10/152) and clinical samples of various subtypes were tested in both assays. The Aptima assay was more sensitive than the COBAS assay.

The good sensitivity, specificity and agreement with other commercial assays make the HIV-1 Quant Dx Assay appropriate for both viral load monitoring and detection of HIV-1 infections.

***Keywords*—**HIV viral load, Aptima, Roche, Panther system.

I. Introduction

DETECTION and quantification of HIV-1 is important not only for diagnosis of HIV-1 infections but also for management of HIV-1 patients [1] and research applications [2], [3]. Quantitative measurements of HIV in the peripheral blood has shown that higher viral loads may be correlated with increased risk of clinical progression of HIV-associated disease, and reductions in plasma virus levels may be associated with decreased risk of clinical progression [4]-[6]. The World Health Organization (WHO) 2013 consolidated guidelines recommends viral load monitoring as the preferred approach compared with immunological and clinical monitoring; viral load monitoring provides an early and more accurate indication of treatment failure and the need to switch to second-line drugs, which leads to reductions in accumulated drug-resistance mutations and to improved clinical outcomes. Measuring viral load can also help to discriminate between treatment failure [7] and non-adherence and can serve as a proxy for the risk of transmission at the population level. Early diagnosis of HIV-1 infection and linkage to care is essential in controlling the HIV epidemic because people with recent infections account for 30-50% of new HIV infections [8], [9]. Because of these benefits there is a concerted effort to support the scaling up of viral load measurement capacity also in resource limited settings. [10], [11].

Despite the multiple commercially available tests for diagnosis of HIV-1 infection (i.e., 4th generation antigen/antibody tests), and for viral load monitoring, none of these tests have regulatory approval for both diagnosis and viral load monitoring, thus requiring a return visit by a patient before treatment can be initiated [12]. The Aptima HIV-1 Quant Dx assay on the Panther system is the first commercially available assay with regulatory approval for both the diagnosis of HIV-1 and viral load monitoring. This assay may enable rapid treatment initiation since its results can be interpreted both qualitatively and quantitatively.

The Aptima HIV-1 Quant Dx assay uses Transcription Mediated Amplification (TMA) and real time detection technologies to detect and quantify HIV-1. TMA utilizes two enzymes, moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase for amplification. The reverse transcriptase is used to generate DNA copies of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from each DNA copy template and this exponential amplification makes the TMA technology ideal for development of sensitive nucleic acid tests. Two regions of HIV-1 RNA (Pol and LTR) are amplified by the Aptima assay. Amplification of these two regions is achieved using specific primers which are designed to amplify HIV-1 groups M, N, and O. The primer design and the dual target approach ensure accurate detection and quantitation of HIV-1. This test uses 0.5 mL of specimen volume per test.

The Panther system is a fully-automated random access nucleic acid analyzer. The system can provide HIV-1 results from primary blood collection tubes directly loaded on the system after centrifugation to separate the plasma, with no further manipulation required. The system generates over 300 test results within an 8 hour shift and the first result is available within 3 hours and subsequent results every 5

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II. Materials and Methods

Nine-hundred and seventy nine specimens selected at random from the routine testing at St Thomas' Hospital London were used in this study. As part of clinical management specimens were tested using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. An aliquot of the same specimen was then anonymized and tested on the Panther system using the Aptima HIV-1 Quant Dx Assay.

Side-by-side testing of 25 and 50 IU/mL panels manufactured by diluting the 3rd international HIV-1 RNA WHO standard (NIBSC code 10/152) into HIV negative plasma was used for comparison of sensitivity of the two assays. Fifteen to thirty replicates of each panel member was tested in each assay for this comparison. Additional testing was conducted with panels composed of HIV-1 Group M subtypes and recombinants (A, B, C, G, A/G) to compare the sensitivity of the Aptima assay to the COBAS assay. Panels were prepared by diluting HIV-1 positive clinical specimens of each subtype in negative plasma to concentrations of 30, 90 and 270 copies/mL. These were tested in replicates of 10 in both the Aptima and COBAS assays. The specificity of the Aptima assay was assessed by testing 600 clinical specimens from HIV-1 negative individuals.

III. Results and Discussion

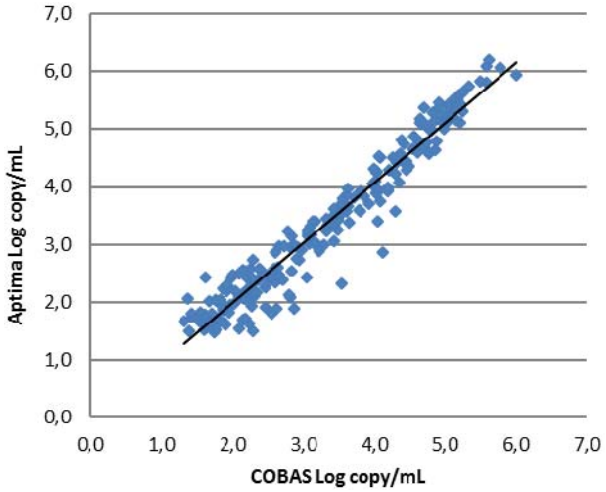

Fig. 1 A Scatter plot comparing the quantitative results from 234 clinical Specimens tested in Aptima HIV-1 Dx assay and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2

From the 979 specimens collected at St Thomas' Hospital London, 234 gave quantitative HIV-1 viral load results in both the COBAS and Aptima assays. A linear regression slope of 1.04 and an intercept of -0.097 were obtained for this comparison showing good agreement between the two assays (Fig. 1). As shown in Fig. 2, Bland Altman analysis of these results also show good agreement between results in the two assays across the assay range.

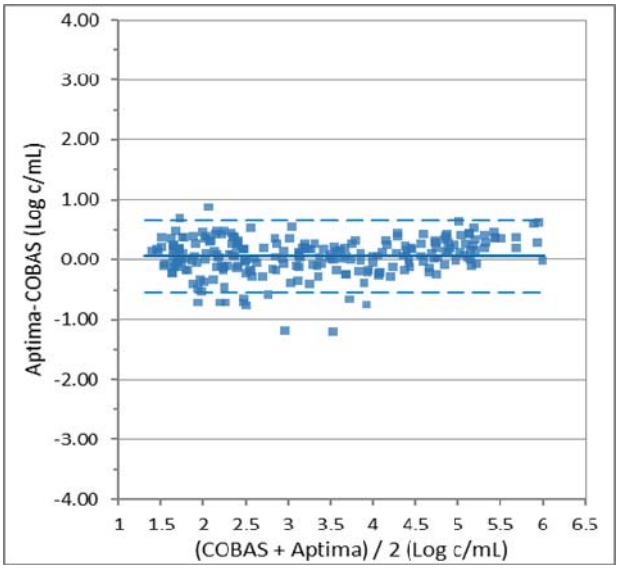

Fig. 2 Bland Altman plot comparing the quantitative results from 234 clinical Specimens tested in Aptima HIV-1 Dx assay and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2

TABLE I
Discordant Analysis of Samples with More Than 1 Log Difference between Roche and Aptima Results on Initial Testing

COBAS V2 (log c/mL)	Abbott (log c/mL)	Aptima initial result (log c/mL)	Aptima Retest (log c/mL)
4.13	3.75	2.86	4.20
3.55	2.62	2.31	Not Retested

Only 2 samples differed in viral load results by more than 1 log between the two assays. These two specimens were tested in the Abbott Real Time HIV-1 assay for discordant analysis. For one of these samples the Aptima result was lower than both the COBAS and Abbott Real Time result. This sample was then retested in Aptima and the retest result was within 0.5 logs of both COBAS and Abbott results. For the other sample the Abbott result was within 0.5 log of the Aptima result (Table I). Other investigators have reported similar discordant rates when comparing commercially available HIV viral load assays [13]-[18].

The 979 specimens collected at St Thomas' Hospital London were categorized as "Target Not Detected" (TND), detected but less than the lower limit of quantitation for the assay (< 20 copies/mL for COBAS and < 30 copies/mL for Aptima) or as "Quant" (giving a quantitative result in the assay). These results are shown in Table II. The results show that 62% (611/979) of the specimens tested gave concordant results (Table II) for the two assays.

TABLE II
Comparison of Results of Nine Hundred and Seventy Nine Specimens Tested in the Aptima HIV-1 Dx Assay and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

		Aptima		
		TND	<30	Quant
COBAS V2	TND	253	151	7
	<20	96	124	14
	Quant	15	85	234

"TND" *Is* "Target Not Detected"

Fifteen specimens gave a quantitative value in the Roche assay but reported target not detected results in the Aptima assay. Seven specimens gave a target not detected result in the COBAS assay but had a quantitative result in the Aptima assay. Part of the reason for this may be that the COBAS assay reports viral load results down to 20 copies/mL (1.30 log copies/ml) but the Aptima assay has a lower limit of quantitation of 30 copies/mL (1.47 log copies/mL). To address this, the data was reanalyzed after applying a lower limit of quantitation of 30 copies/mL to both assays (Table III). This increased the agreement between the two assays from 62 to 65%. This also reduced the number of specimens with target not detected results in the Aptima assay and a quantified result in COBAS from 15 to 8. In addition, the number of specimens that were quantified in the COBAS assay and detected in the Aptima assay was reduced from 85 to 60. This demonstrates that many of the quantified specimens that categorized differently had viral loads close to the limit of quantitation of the assays.

TABLE III
COMPARISON OF RESULTS OF NINE HUNDRED AND SEVENTY NINE PATIENT PLASMA SPECIMENS TESTED IN THE APTIMA HIV-1 DX ASSAY AND THE COBAS® AMPLIPREP/COBAS® TAQMAN® HIV-1 TEST, V2.0

		Aptima		
		TND	<30	Quant
COBAS V2	TND	253	151	7
	<30	103	149	19
	Quant	8	60	229

"TND" *Is* "Target Not Detected"

In this clinical sample set there were more specimens that gave a detectable result in the Aptima assay compared to COBAS (151 vs 103). Similar results have been observed by other investigators on comparing the sensitivities of different HIV viral load assays [19-21]. It should be noted that the sensitivity studies documented in the package insert for the COBAS assay were performed with the 2nd International HIV-1 WHO standard (NIBSC code 97/650) while those for Aptima were conducted with the 3rd international HIV-1 WHO standard (NIBSC code 10/152). A direct comparison using the 3rd international standard for HIV-1 diluted to 25 and 50 IU/mL and tested in both assays show that Aptima had a higher reactivity rate than COBAS at both concentrations (Table IV).

Additional testing conducted with subtype panels made from clinical specimens show that the Aptima assay detected 100% of samples at 30, 90 and 270 copies/mL (10 replicates of each panel). The COBAS assay failed to detect 1 replicate at 30 and 90 c/mL for subtype B, 4 replicates at 30 and 1 replicate at 90 c/mL for subtype G (Table V). Therefore the COBAS assay was less sensitive than the Aptima assay with both WHO standards and panels made from clinical specimens of multiple HIV-1 subtypes.

Another possible explanation for the difference in detection rate between the two assays is that the Aptima assay could be reporting more false positive results. To rule this out a specificity study was conducted by testing 600 plasma samples from HIV-1 negative patients. The specificity of the Aptima assay was 99.83% (Table VI). There was 1 false positive result with "<30 c/ml detected" result on initial testing that gave target not detected results on retesting in Aptima. This demonstrates that the Aptima assay has good specificity and sensitivity. The 151 clinical specimens that gave detected results in Aptima but not in COBAS (Tables II and III) are therefore not false positive but low level positives from HIV-1 patients on anti-retroviral therapy.

TABLE IV
COMPARISON OF SENSITIVITY BETWEEN COBAS AND APTIMA ASSAYS WITH 3RD HIV WHO STANDARD 10/152 DILUTED TO 25 AND 50 IU/ML IN PLASMA

Concentration in IU/mL	Assay	# Replicates tested	# Positive	% Reactivity
25	Roche	15	9	**60**%
	Aptima	30	27	90%
50	Roche	15	12	**80**%
	Aptima	30	30	100%

TABLE V
REACTIVITY OF HIV-1 SUBTYPE PANELS AT 30, 90 AND 270 C/ML ON TESTING IN ROCHE AND APTIMA ASSAYS

HIV-1 Subtype	Assay	HIV-1 Concentration c/mL		
		30	90	270
A	COBAS	100%	100%	100%
	Aptima	100%	100%	100%
B	COBAS	**90%**	**90%**	100%
	Aptima	100%	100%	100%
C	COBAS	100%	100%	100%
	Aptima	100%	100%	100%
G	COBAS	**40%**	**90%**	100%
	Aptima	100%	100%	100%
A/G	COBAS	100%	100%	100%
	Aptima	100%	100%	100%

TABLE VI
SPECIFICITY TESTING FOR THE APTIMA ASSAY WITH 600 HIV-1 NEGATIVE PLASMA SPECIMENS TESTED WITH 2 REAGENT LOTS

Aptima Reagent lot	Number of individual patient plasma samples tested	Number TND results in Aptima	Aptima Specificity (95% Confidence Interval)
1	300	299	99.67
2	300	300	100.00
Total	600	599	99.83 (99.06-99.97)

IV. CONCLUSION

The Aptima HIV-1 Quant Dx Assay gave comparable viral load results to the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 for most clinical samples. However the sensitivity of the Aptima Assay was higher than the COBAS v2.0 assay on testing clinical samples, dilutions of NIBSC WHO standards and sensitivity panels belonging to various HIV-1 subtypes. The Aptima assay also has good specificity. The high sensitivity and specificity of the Aptima assay and good agreement in quantitation results with other commercial assays makes it a good candidate for both diagnosis of HIV-1 infections and viral load monitoring.

REFERENCES

[1] Mellors, J.W., C. R. Rinaldo, Jr, P. Gupta , R. M. White, J. A. Todd , L. A. Kingsley 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272:1167–1170.

[2] Wei X., S. K.Ghosh, M.E. Taylor , V. A. Johnson , E. A. Emini, P. Deutsch , J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. H. Hahn. 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373:117–122.

[3] Perelson A.S., A. U. Neumann, M. Markowitz, J. M. Leonard, D.D. Ho 1996. HIV-1 dynamics *in vivo*: Virion clearance rate, infected cell life-span, and viral generation time. Science 271:1582–1586

[4] O'Brien, W. A., P. M. Hartigan, D. Martin, J. Esinhart, A. Hill, S. Benoit, M. Rubin, M. S. Simberkoff, and J. D. Hamilton. 1996. Changes in plasma HIV-1 RNA and CD4 lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. N. Engl. J. Med. 334:426-431.

[5] Welles, S. L., J. B. Jackson, B. Yen-Lieberman, L. Demeter, A. J. Japour, L. M. Smeaton, V. A. Johnson, D. R. Kuritzkes, R. T. P. A. Reichelderfer, D. D. Richman, R. Reichman, M. Fischl, R. Dolin, R. W. Coombs, J. O. Kahn, C. McLaren, J. Todd, S. Kwok, and C. S. Crumpacker. 1996. Prognostic value of plasma human immunodeficiency virus type I (HIV-1) RNA levels in patients with advanced HIV-1 disease and with little or no zidovudine therapy. AIDS Clinical Trials Group Protocol 116A/116B/117 Team. J. Infect. Dis. 174:696-703.

[6] Coombs, R. W., S. L. Welles, C. Hooper, P. S. Reichelderfer, R. T. D'Aquila, A. J. Japour, V. A. Johnson, D. R. Kuritzkes, D. D. Richman, S. Kwok, J. Todd, J. B. Jackson, V. DeGruttola, C. S. Crumpacker, and J. Kahn. 1996. Association of plasma human immunodeficiency virus Type I RNA level with risk of clinical progression in patients with advanced infection. AIDS Clinical Trials Group (ACTG) 116B/117 Study Team. ACTG Virology Committee Resistance and HIV-1 RNA Working Groups. J. Infect. Dis. 174:704-712.

[7] Human immunodeficiency virus type 1 RNA level and CD4 count as prognostic markers and surrogate end points: a meta-analysis. HIV Surrogate Marker Collaborative Group. AIDS Res Hum Retroviruses. 2000;16 (12):1123-1133.

[8] Brenner B.G., M. Roger , J.P. Routy, D. Moisi, M. Ntemgwa, C. Matte, J. G. Baril, R. Thomas, D.Rouleau, J. Bruneau, R. Leblanc, M. Legault, C. Tremblay, H. Charest, M. A. Wainberg. Quebec Primary HIV Infection Study Group. 2007 High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis;195: 951-959.

[9] Cohen M.S., Y.Q. Chen, M. McCauley, T. Gamble, M. C. Hosseinipour, N. Kumarasamy, J.G. Hakim, J. Kumwenda, B. Grinsztejn, J.H.S. Pilotto, S.V. Godbole, S. Mehendale, S. Chariyalertsak, B.R. Santos, K.H. Mayer, I.F. Hoffman , S.H. Eshleman, E. Piwowar-Manning, L. Wang, J. Makhema, L.A. Mills, G. de Bruyn, I. Sanne, J. Eron, J. Gallant, D. Havlir, S. Swindells, H. Ribaudo, V. Elharrar, D. Burns, T. E. Taha, K. Nielsen-Saines, D. Celentano, M. Essex, and T.R. Fleming for the HPTN 052 Study Team Prevention of HIV-1 infection with early antiretroviral therapy. 2011 Prevention of HIV-1 Infection with Early Antiretroviral Therapy. N Engl J Med.; 365(6):493-505.

[10] WHO. 2013. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. World Health Organization, Geneva, Switzerland. http://www.who.int/hiv/pub/guidelines/arv2013/en/

[11] WHO. 2014. March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. World Health Organization, Geneva, Switzerland. http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013upplement_march2014/en/

[12] Bennett B, S. Fordan, L. Haddock-Morilla, M. Rowlinson 2013 HIV Single staging algorithm: integration and maximization of resources by reducing time between HIV diagnosis and treatment. J. Clin.Virol 58(1):34-37.

[13] Styer L.S., T.J. Sullivan, M.M. Parker 2011. Evaluation of an alternative supplemental testing strategy for HIV diagnosis by retrospective analysis of clinical HIV testing data Journal Clin. Virol 52 (1): S35– 40

[14] Amendola A., P. Marsella, M. Boisi, F. Forbici, C. Angeletti, M. R. Capobianchi. 2014. Ability of two commercially available assays (Abbott RealTime HIV-1 and Roche AmpliPrep/Cobas Taqman HIV-1 Version 2.0) to quantify low HIV-1 RNA levels (<2,000 copies/millileter); Comparison of clinical samples and NIBSC working reagents for nucleic acid testing assays. J. Clin. Microbiol. 52(6): 2019-2026

[15] Sire J.M., M. Vray, M. Merzouk, J.C. Plantier, J. Pavie, S.Maylin, J. Timsit, C. Lascoux-Combe, J. Molina, F. Simon and C.Delaugerre. 2011 Comparative RNA quantification of HIV-1 Group M and Non M with the Roche Cobas AmpliPrep/Cobas Taqman HIV-1 v2.0 and Abbott RealTime HIV-1 PCR Assays. J. Acquir. Immune Defic. Syndr. 56(3): 239-242.

[16] Karasi J.C., F. Dziezuk, L. Quennery, S. Forster, U Reischl, G. Colucci, D. Schoener, C. Seguin-Devaux, J.C Schmit. 2011. 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. J. Clin. Virol. 52: 181-186.

[17] Swenson L.C., B. Cobb, A.M. Geretti, P.R. Harrigan, M. Polijak, C. Seguin-Devaux, C. Verhofstede, M. Wirden, A. Amendola, J. Boni, T. Bourlet, J.B. Huder, J.C. Karasi, S. Zidovec Lepej, M.M. Lunar, O. Mukabayire, R. Schuurman, J. Tomažič, K. Van Laethem, L. Vandekerckhove, and A. M. Wensing; International Viral Load Assay Collaboration 2014 Comparative Performances of HIV-1 RNA Load Assays at Low Viral Load Levels: Results of an International Collaboration J. Clin. Microbiol 52(2):517-523.

[18] Pyne M. T., A. Wilson, D.R. Hilliyard. 2012. Large-scale comparison of Roche Cobas AmpliPrep/cobas TaqMan and Abbott RealTime HIV assays. J. Virol. Methods 184: 106-108.

[19] Gatanaga H, K., Tsukada, H. Honda, J. Tanuma, H. Yazaki, T. Watanabe, M. Honda, K. Teruya, Y. Kikuchi and S. Oka 2009 Detection of HIV type 1 load by the Roche Cobas TaqMan assay in patients with viral loads previously undetectable by the Roche Cobas Amplicor Monitor. Clin Infect Dis 48:260–2.

[20] Rebeiro P.F., A. Kheshti, S. S. Bebawy, S. E. Stinnette, H. Erdem , Y.Tang, T. R. Sterling, S.P. Raffanti, R. T. D'Aquila 2008. Increased Detectability of Plasma HIV-1 RNA after Introduction of a New Assay and Altered Specimen-Processing Procedures Clin Infect Dis. 47 (10): 1354-1357.

[21] Rensburg E.J., K. Tait, A. Watt, R. Schall. 2011. Comparative Evaluation of Roche Cobas Ampliprep/Cobas Tqman HIV-1 version 2 Test using TaqMan 48 Analyzer and the Abbott RealTime HIV-1 Assay. J. Clin. Microbiol 49(1):377-379.