Comparison of the new Hologic Aptima HIV-1 Quant Dx Assay with the established Abbott RealTi*me* in HIV-1 viral load measurement

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Introduction and Purpose:
Hologic's Aptima HIV-1 Quant Dx assay is a HIV-1 RNA quantitative assay based on real-time Transcription Mediated Amplification (TMA) that runs on the fully automated Panther system with random access. A comparison with the Abbott m2000 RealTi*me* assay was performed. Special focus was put on linearity (in three different common subtypes), viremia and precision near the limit of detection of the assays, non-B subtypes, and integrase inhibitor resistant samples.

Methods
Fresh (n=282), frozen (non-B, n=117; with integrase mutations, n=22) and diluted (n=559) patient samples spread over the clinical relevant range of viral load were tested. The Aptima assay is a dual target assay with targets in pol (integrase) and LTR-region, whereas RealTi*me* uses a single target in the integrase region. Samples with integrase mutations were additionally quantified with the Roche Cobas TaqMan v2.0, with targets outside the integrase region. For linearity comparison we set up and optimized two linear models minimizing the residual sum of squares (RSS) for each set of log-scaled viral loads measured by Aptima respectively m2000.

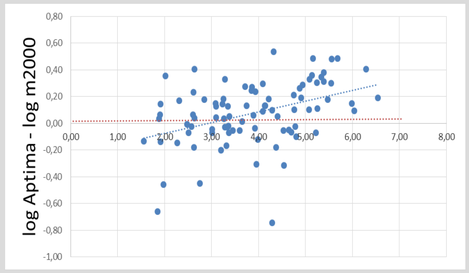

Figure 1: Bland Altman
mixed subtypes, preselected frozen samples

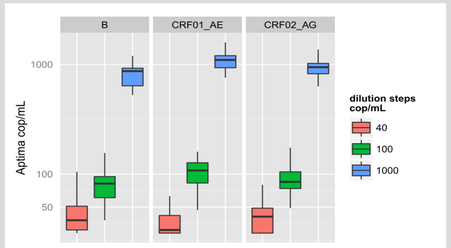

Figure 3: Inter-assay variance
three subtypes in three dilutions and in 21 independant runs

Table 1: **Correlation in clinical samples of HIV-1 seropositives**
Not preselected fresh clinical routine samples (n=100)

		Aptima			
		not detected	<30 c./mL	quantificated	total
m2000	not detected	40	25	2	67
	<40 c./mL	1	3	2	6
	quantificated	0	2	25	27
	total	41	30	29	100

Table 2: **Intra-assay variance**
Coefficients of variation in low viral load range

Viral Load	Assay	CRF02_AG	B	CRF01_AE
1.7 log c./mL	Aptima	6.8%	6.9%	8.4%
	m2000	9.5%	8.2%	7.5%
2.0 log c./mL	Aptima	7.7%	6.9%	4.0%
	m2000	7.2%	4.7%	5.9%
2.3 log c./mL	Aptima	5.4%	5.0%	5.1%
	m2000	4.3%	4.0%	3.0%

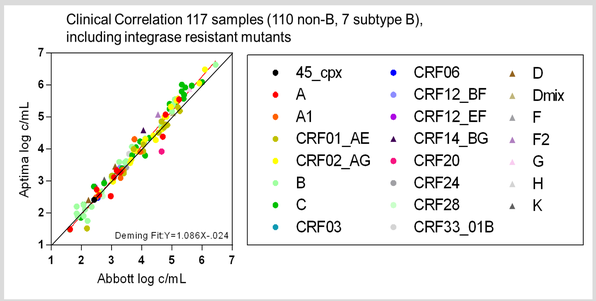

Figure 2: Correlation of diverse subtypes, Deming regression frozen preselected samples

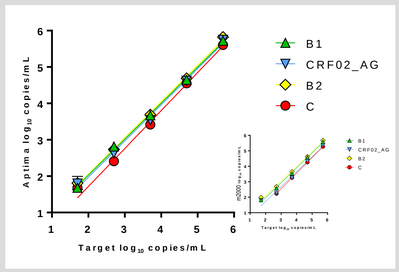

Figure 4: Linearity Aptima and m2000
4 samples, 3 subtypes, 5 dilutions, 5 replicates

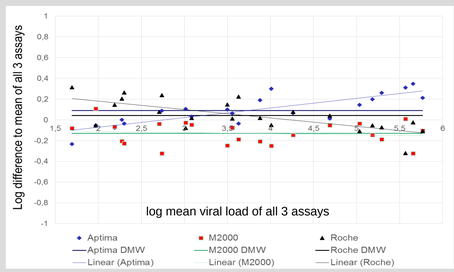

Figure 5: Integrase resistant samples, n=22
DMW = difference to mean out of all three assays

Results
Aptima HIV-1 Quant Dx assay showed excellent performance in high throughput, routine use even in samples with low viremia or with mutations in the integrase region. With a lower limit of quantification (LLOQ) of 30 cps/mL and a lower limit of detection (LLOD) of 13 cps/mL, the Aptima assay classified more samples as "detected" (30 versus 6) than the RealTi*me* assay in 100 unselected fresh samples (Tab. 1). Bland Altman plots demonstrated high concordance between the two assays (Fig. 1 and 5). The mean difference was below 0.1 log cps/mL, a trend of same values in lower levels and higher quantification in higher viral loads could be observed. High concordance was also shown for non-B subtypes (Fig. 2). Intra- and inter-assay variation was low and comparable to RealTi*me* with intra-assay %CV ranging from 4.0% for samples with a viral load of 2.0 log cps/mL to 8.4% with 1.7 log cps/mL (Tab. 2 and Fig. 3). Linearity was shown by serial dilution (subtype B 2 different samples, C and CRF02_AG) from 5.7 log cps/mL to 1.7 log cps/mL (Fig. 4). Calculation yielded RSS values of 0.967 for Abbott and 0.944 for Aptima, testing for difference of both models resulted in a p-value <0.05 indicating values measured by Aptima are characterised slightly more linear than Abbott samples. Mutations associated with resistance in the integrase region and up to 22 differences to consensus B sequence were not found to impact results in the m2000 nor in the Aptima assay as compared to the Roche assay (Fig. 5).

Conclusions
The Aptima HIV-1 Quant Dx assay showed excellent correlation with RealTi*me* with high sensitivity (trend to superiority), linearity over the whole range of interest and accuracy in the therapeutic relevant range for all tested HIV-1 subtypes. With random access, the ability to continuously load samples and time to first result of about 150 minutes this assay is a major improvement in the viral load monitoring of HIV-1 infection.

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