

Comparison of the Hologic Aptima HIV-1 Quant Dx Assay with the Abbott RealTime and Roche Cobas 6800 in simultaneously HIV-1 viral load measurement focussing low end detection

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Introduction and Purpose:

There is still an extensive discussion on comparability of different common assays in the quantification of low level viral loads in monitoring HIV-1 infected patients. Quantifiable results above detection limit can be a hint for therapeutic difficulties or viral breakthrough and are disturbing patients and physicians. There were some hints that the cobas 6800 platform quantifies more samples as so called blips than competitive systems. We compared Hologic Aptima HIV-1 Quant Dx, the Abbott m2000 RealTime and the Roche HIV-1 assay on the cobas 6800 platform. All measurements were performed on the same day with fresh unfrozen and separated plasma focussing low end performance and comparability.

Methods

Fresh samples (n=1011) from routine monitoring of patients were tested. Plasma was attained by centrifugation directly after arrival at MIB laboratory. The only criteria for inclusion was sufficient plasma volume to test all three assays. RealTime and Aptima were tested batch-wise at MIB and cobas 6800 testing was performed at Labor 28, Berlin. Samples were transported twice daily to "Labor 28" to ensure real side-by-side comparison. Results were compared in three groups: "not detected", "detected" and "quantifiable" with individual lower limits of quantification (LLOQ; RealTime 40 c/mL, Aptima 30 c/mL and cobas 6800 20 c/mL). All results were also compared using the clinically defined cut off of 50 c/mL and additionally focussing the low viral load range between 50 and 250 c/mL. Overall concordance was analysed by Deming-regression and Bland-Altman plots.

Results

Concordance by defining the viral load levels as undetectable, "detectable, \leq LLOQ" and quantifiable between the three systems differed depending on the cut-off chosen. With system specific cut-offs nearly twice as much quantifiable results (234 vs 136 and 140) were reported with the 6800 assay compared to RealTime and Aptima, respectively. "Detectable, \leq LLOQ" results were highest in Aptima (359) followed by 6800 (271) and RealTime (163). Using the clinically established cut-off of 50 c/mL reduces the differences between the assay results. 124 vs 122 and 136 quantifiable results were reported with RealTime, Aptima and 6800, respectively. This translates to 12.3%, 12.1% and 13.5% of all tested routine samples or 1.4% more quantified samples by 6800 compared to Aptima. Regarding the low viral range between 50 and 250 c/mL we found the same descending order of quantified samples with most samples in this range with cobas 6800 (n=72) followed by RealTime (n=65) and Aptima (n=58). 95 samples were quantified by all three assays (cut-off 50 c/mL), 12 only by Aptima and 6800, 7 only by 6800 und RealTime, and 4 with Aptima and RealTime only. In Deming regression all comparisons lead to r-values above 0.98 and Bland-Altman analysis showed the lowest mean difference in log copies per mL for 6800/Aptima (0.038), followed by 6800/RealTime (0.103) and Aptima/RealTime (0.168).

Conclusions

All three assays performed with a high concordance. Taking the test-specific cut-offs 6800 quantified nearly twice as much directly discharged plasma samples than Aptima and RealTime. Switching to the clinical validated cut-off of 50 c/mL the difference in quantified samples by cobas 6800 was reduced to only 1.4% or 1.2% more in comparison to Aptima and RealTime, respectively. Using primary tubes with the plasma left on top of the cuor the amounts of quantified samples might rise. A possible explanation for higher difference in detecting low level viral loads by cobas 6800 reported by other groups. The two more recently launched assays, Aptima and 6800, showed the best correlation and lowest difference in Bland-Altman analyses.

Concordance in defining viral load not detected, detected, quantified

	RealTime	Aptima	Cobas 6800
not det.	712	512	506
det. \leq LLOQ	163	359	271
quant.	136	140	234
Total	1011	1011	1011

Concordance in defining viral load \geq 50 copies/ml

	RealTime	Aptima	Cobas 6800
not det.	712	512	506
det. \leq 50	175	377	369
quant. $>$ 50	124	122	136
Total	1011	1011	1011

$>$ 50 copies/ml
 Aptima: 12,1%
 RealTime: 12,3%
 Roche 6800: 13,5%

Concordance in defining the HIV-1 viral load levels as undetectable, detectable and quantifiable between Aptima, Cobas 6800 and RealTime with individual and adjusted thresholds

Cobas 6800	Aptima				Total
	NEG	$<$ 30	$>$ 30	ULQ	
NEG	374	130	2	0	506
$<$ 20	125	140	6	0	271
$>$ 20	13	89	131	0	233
ULQ	0	0	0	1	1
Total	512	359	139	1	1011

weighted kappa: 0.53 Agreement: 63.9%

Cobas 6800	Aptima (threshold adjusted)				Total
	NEG	$<$ 30	$>$ 30	ULQ	
NEG	374	130	2	0	506
$<$ 30	132	181	13	0	326
$>$ 30	6	48	124	0	178
ULQ	0	0	0	1	1
Total	512	359	139	1	1011

weighted kappa: 0.56 Agreement: 67.3%

RealTime	Aptima				Total
	NEG	$<$ 30	$>$ 30	ULQ	
NEG	457	242	13	0	712
$<$ 40	48	97	18	0	163
$>$ 40	7	20	108	0	135
ULQ	0	0	0	1	1
Total	512	359	139	1	1011

weighted kappa: 0.50 Agreement: 65.6%

RealTime	Aptima (threshold adjusted)				Total
	NEG	$<$ 40	$>$ 40	ULQ	
NEG	457	244	11	0	712
$<$ 40	48	108	12	0	163
$>$ 40	7	20	108	0	135
ULQ	0	0	0	1	1
Total	512	367	131	1	1011

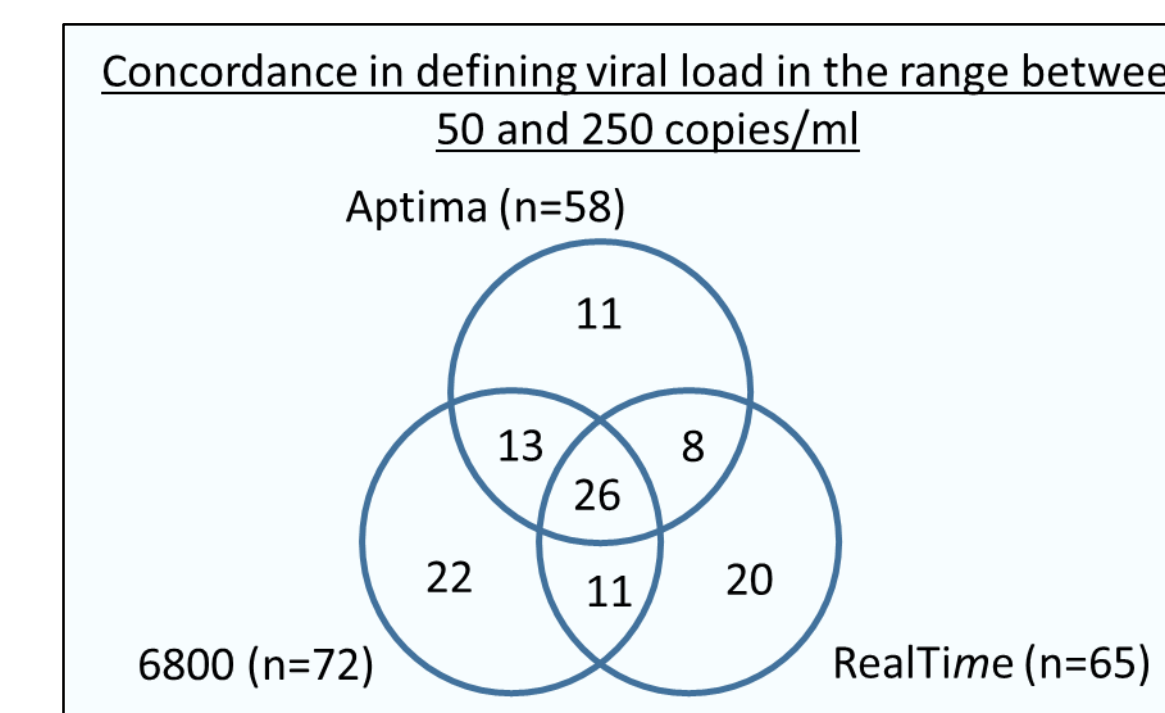
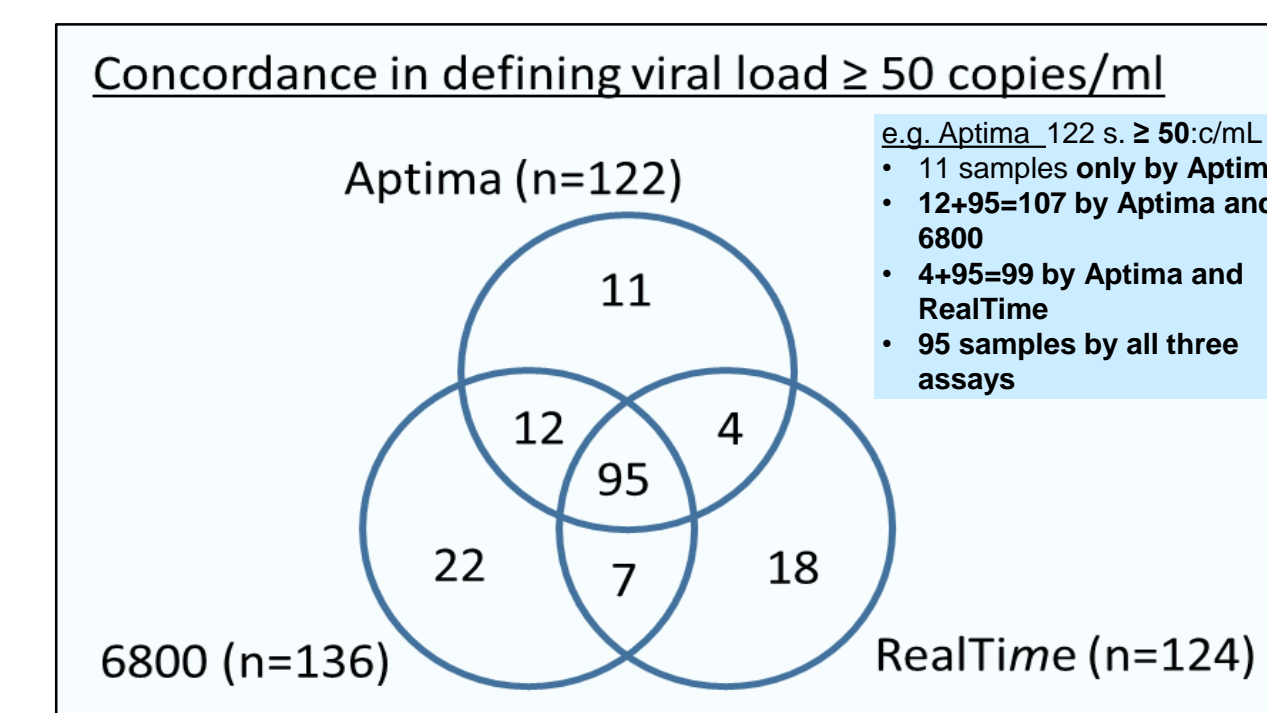
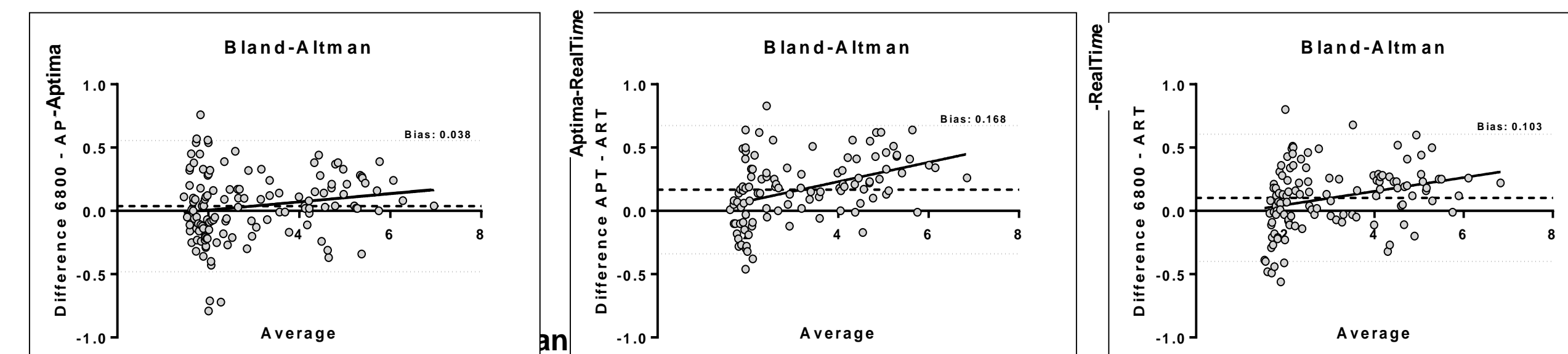
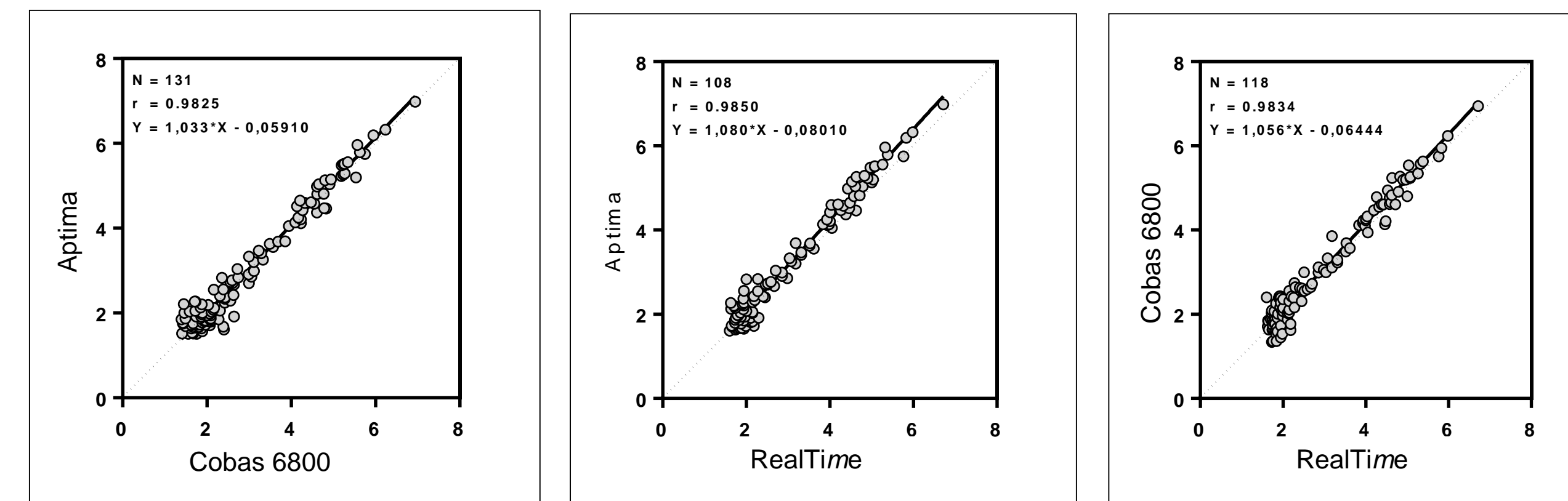
weighted kappa: 0.51 Agreement: 66.2%

Cobas 6800	RealTime				Total
	NEG	$<$ 40	$>$ 40	ULQ	
NEG	445	54	7	0	506
$<$ 20	203	58	10	0	271
$>$ 20	64	51	118	0	233
ULQ	0	0	0	1	1
Total	712	163	135	1	1011

weighted kappa: 0.44 Agreement: 61.5%

Cobas 6800	RealTime (threshold adjusted)				Total
	NEG	$<$ 40	$>$ 40	ULQ	
NEG	445	54	7	0	506
$<$ 40	251	87	10	0	348
$>$ 40	16	22	118	0	156
ULQ	0	0	0	1	1
Total	712	163	135	1	1011

weighted kappa: 0.49 Agreement: 64.4%



RealTime	Aptima	Frequency	6800	Aptima	Frequency	6800	RealTime	Frequency
$<$ 50	$<$ 50	864	$<$ 50	$<$ 50	860	$<$ 50	$<$ 50	853
$<$ 50	\geq 50	23	$<$ 50	\geq 50	15	$<$ 50	\geq 50	22
\geq 50	$<$ 50	25	\geq 50	$<$ 50	29	\geq 50	$<$ 50	34
\geq 50	\geq 50	99	\geq 50	\geq 50	107	\geq 50	\geq 50	102

wkappa: 0.78 wkappa: 0.80 wkappa: 0.75