Hologic Aptima HIV-1 Quant Dx Assay Compared to Abbott RealTi*me* in B and non-B Subtypes in daily routine testing

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Background:

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 RealTime 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.
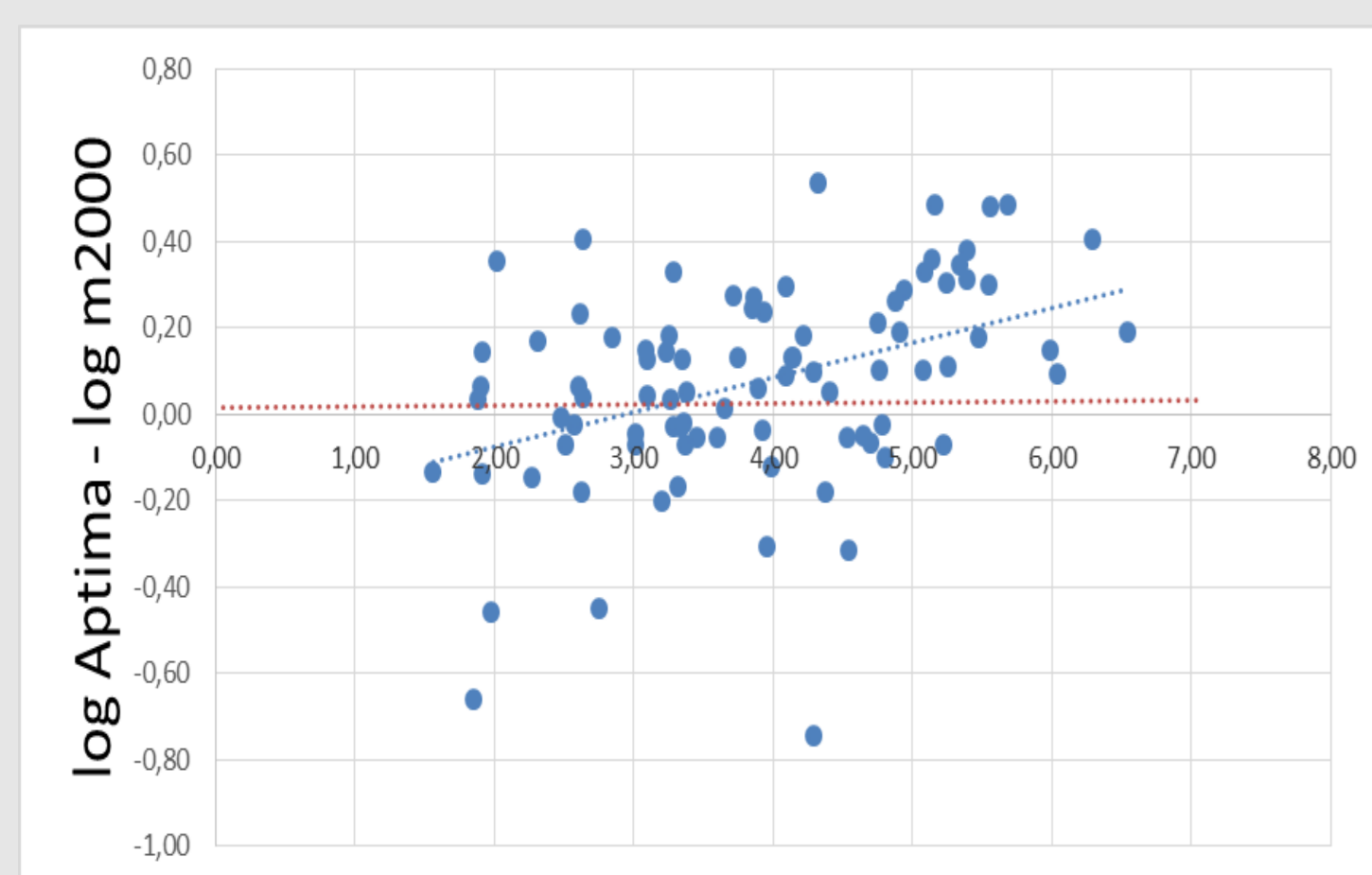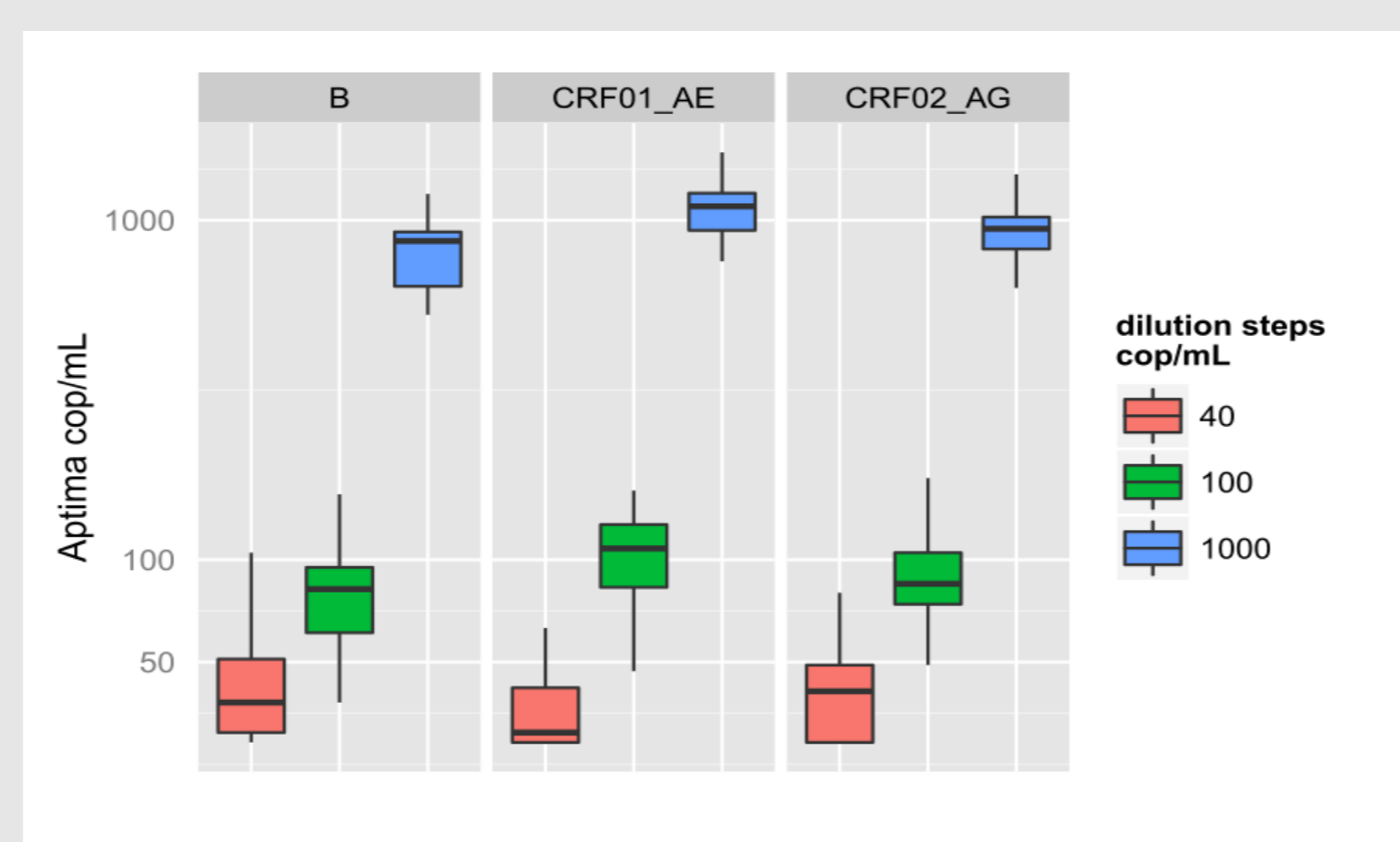
Methods

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.

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. 3). 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. 2). Linearity was shown by serial dilution (subtype B two 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 CAP/CTM (Roche) assay (Fig. 5). In a workflow analysis of a batch of 96 samples including controls the Aptima assay showed a reduced hand-on-time with a time to first result in 150 minutes and all results after 5 hours 3 minutes. A substantial reduction compared to Abbott m2000 and without any engagement in between, a big advantage in planning the technicians shift (Fig. 6).
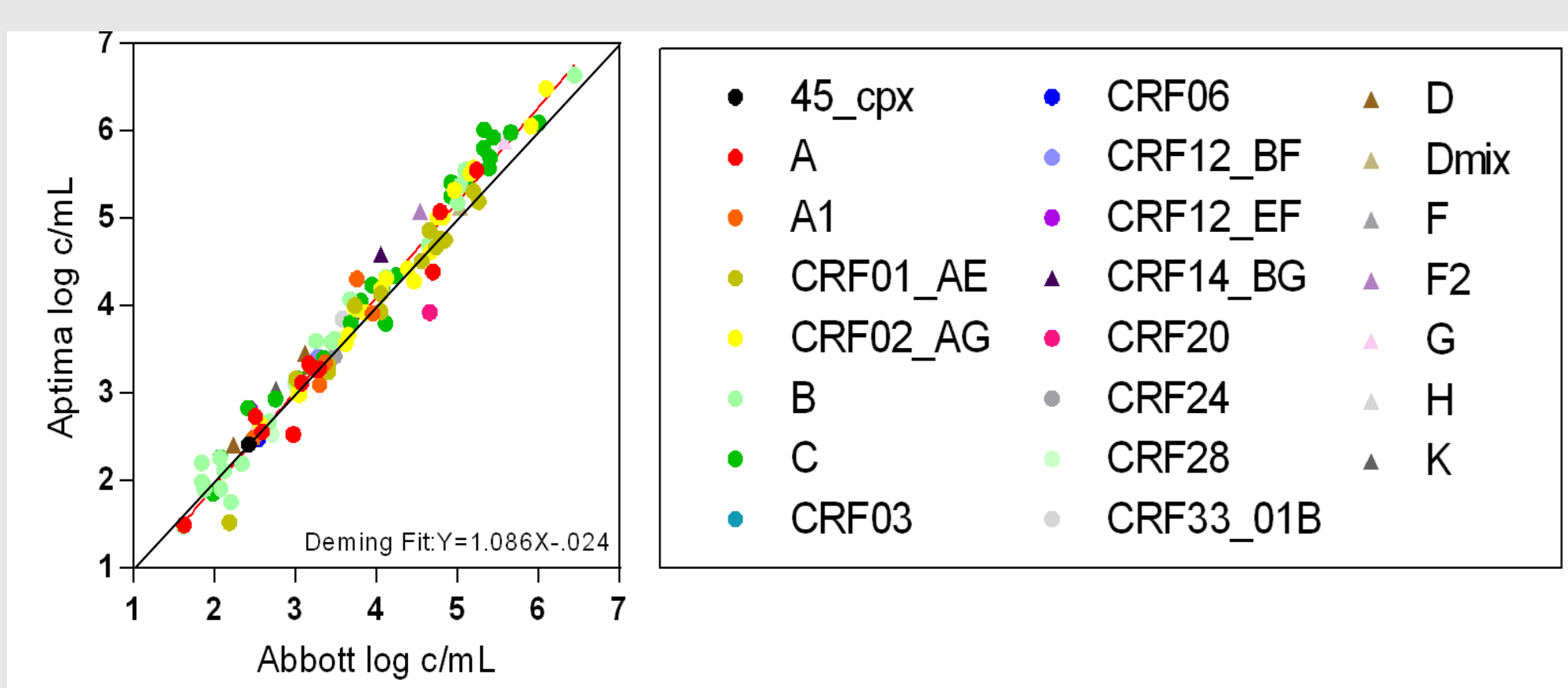

Figure 1: Bland Altman
mixed subtypes, preselected frozen samples

Figure 2: Inter-assay variance
three subtypes in three dilutions and 21 independant runs

Figure 3: Deming regression
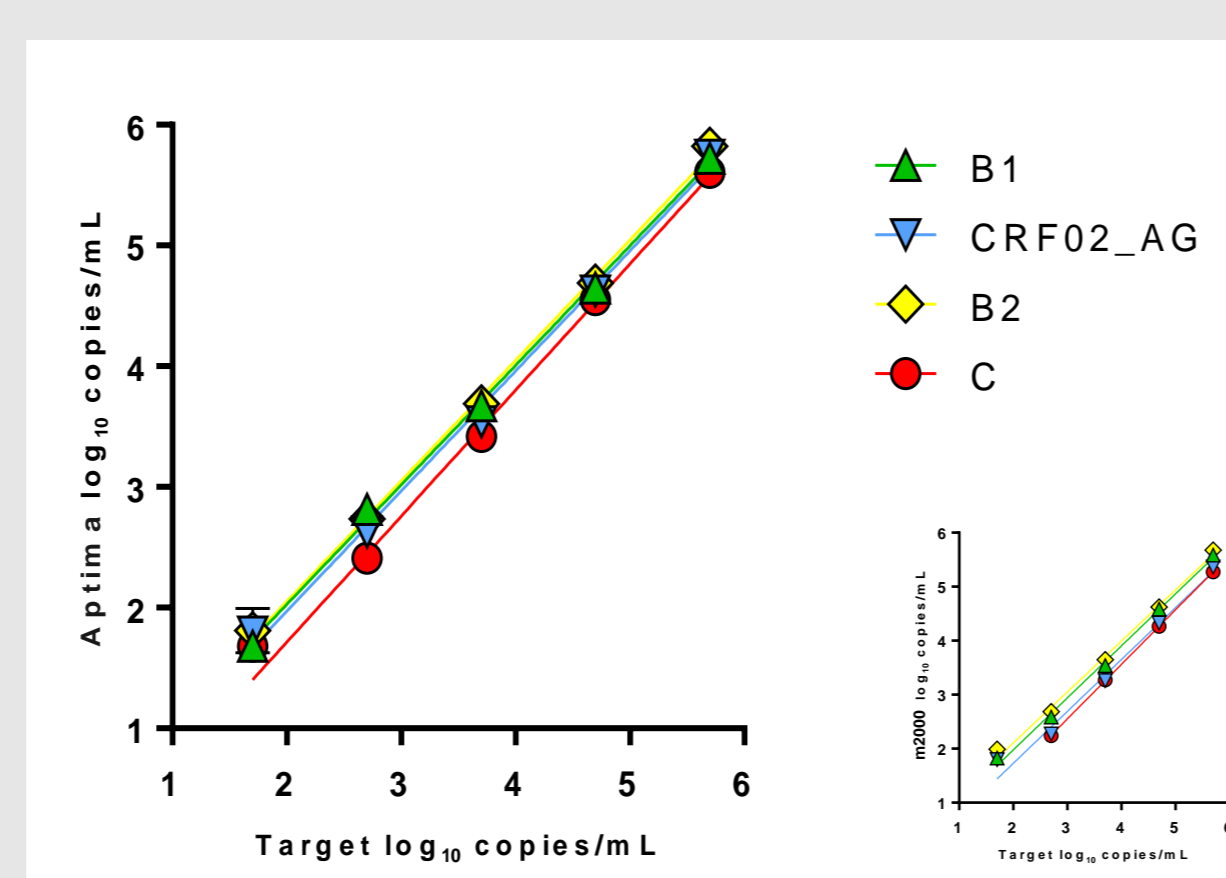Correlation of diverse subtypes, 117 frozen preselected samples

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

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

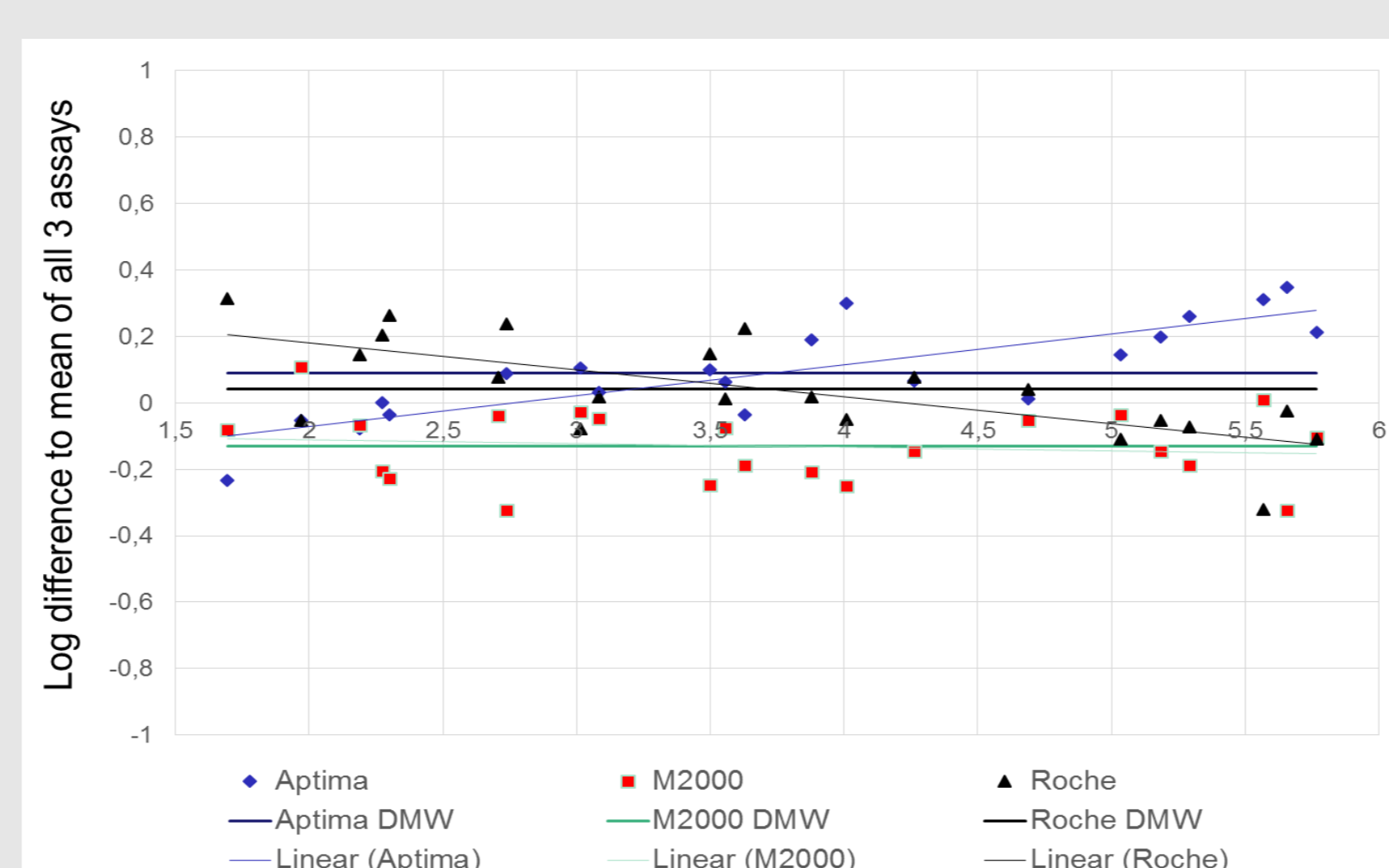| Viral Load	Assay	CRF02_AG	B	CRF01_AE
1.7 log c./mL	Aptima	6.8%	6.9%	8.4%
	m2000	9.5%	8.2%	7.5%
2.0 log c./mL	Aptima	7.7%	6.9%	4.0%
	m2000	7.2%	4.7%	5.9%
2.3 log c./mL	Aptima	5.4%	5.0%	5.1%
	m2000	4.3%	4.0%	3.0%

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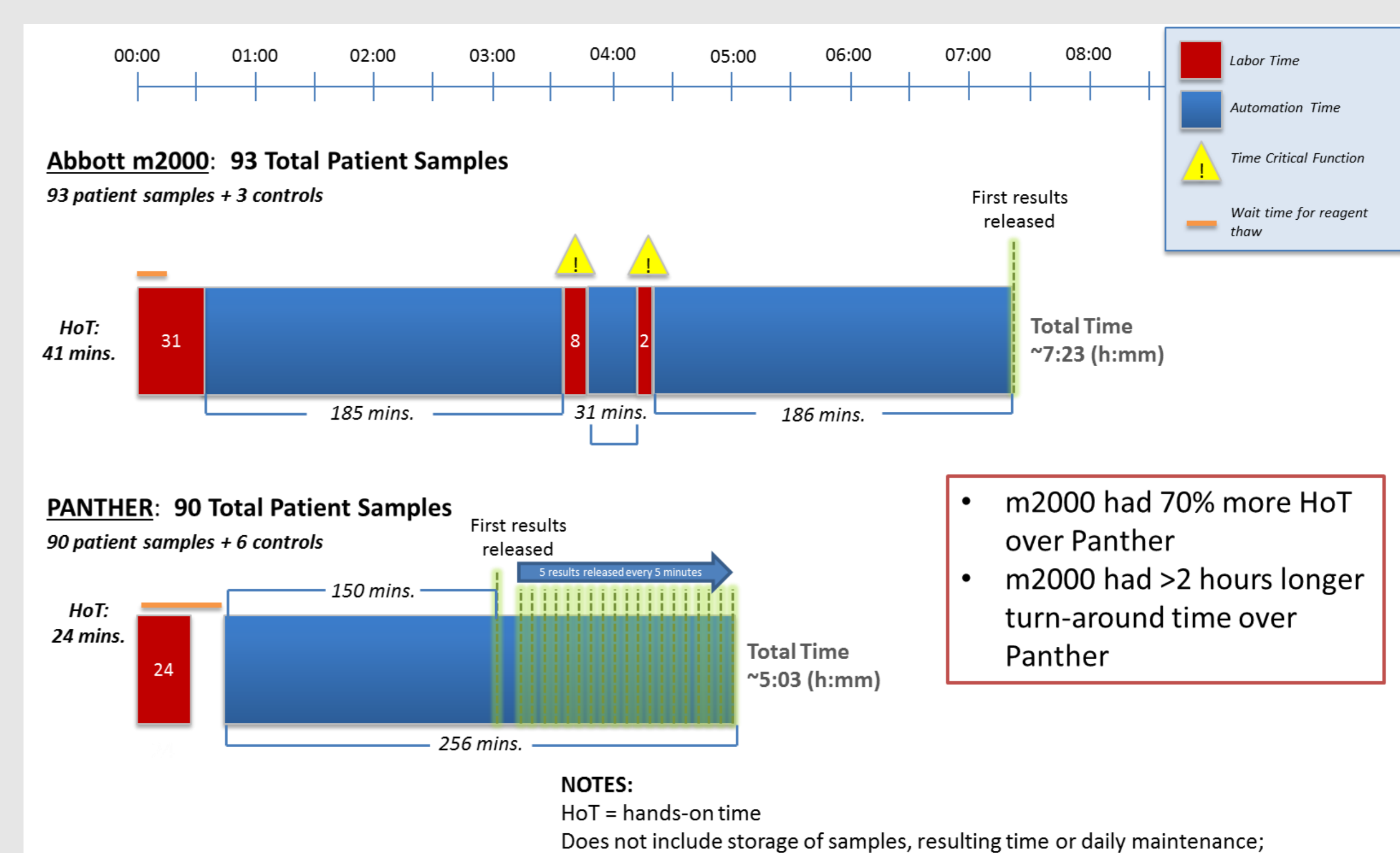DMW = difference to mean out of all three assays

Figure 6: workflow analysis and operational timeline
for batchwise 96 measurements

Conclusions

The Aptima HIV-1 Quant Dx assay showed excellent correlation with RealTi*me* HIV-1 with high sensitivity, linearity and accuracy in the therapeutic relevant range for all tested HIV-1 subtypes. Mutations in the integrase gen did not lead to mal-quantification actually not to a switch to quantification by the second probe in LTR-region. With random access, the ability to continuously load samples and time to first result of 150 minutes it is a major improvement in the viral load monitoring of HIV-1 infection.

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