CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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cobas® 6800 HIV-1 Viral Load Assay
- New Platform -

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Ordering Information and Suggested Codes:

Mnemonic	**HIV VL**
Test Name	HIV 1 Real Time PCR
Test Number	3399905
Specimen	K-3/K-2 EDTA Plasma Requested volume 3 mL, Minimum volume accepted 1.5 mL. Preferably no false-bottom tubes.
Stability	Upon plasma separation: **up to 6 days refrigerated at 2°C to 8°C**, up to 12 weeks frozen at ≤ -18°C. **Note: separate plasma from cells within 24 hours of collection.**
LOINC Codes	70241-5
CPT Codes	87536

Effective Date: Testing offered beginning on Monday, June 5th, 2017.

Performed: Monday through Friday, dayshift

Reference Range:

Age Range	
All	≤ 19 cp/mL

Background:

Quantitative measurements of HIV viremia in the plasma have shown that higher virus levels are correlated with more rapid clinical progression of HIV disease. Furthermore, nearly two decades of clinical research have established that reductions in plasma virus levels with the use of antiretroviral therapy (ART) significantly decrease the risk of clinical progression, including death, development of AIDS, opportunistic infections, and HIV-associated morbidity. HIV viral load is also predictive of the risk of transmission of HIV, and randomized controlled clinical trials have established that early initiation of ART with suppression of the viral load reduces HIV transmission by 96%.

Principle of Test:

Roche Molecular Systems, Inc. **cobas®** HIV-1 is a quantitative test performed on the **cobas®** 6800 System and enables the detection and quantitation of HIV-1 RNA in EDTA plasma of infected patients. Two probes are used to detect and quantify, but not discriminate group M, N and O subtypes. **cobas®** HIV-1 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection.

Validation Data:

Precision Studies:

Within-run and between-run precision were evaluated at two levels, low and high. Patient samples with low-level viral loads were used for the low pool (target 3 to 4 $\log_{10}$ cp/mL), and patient samples with high-level viral loads were used for the high pool (target >5 $\log_{10}$ cp/mL).

For within run precision, ten aliquots of each of the two pools were tested on a single run. For between run precision, five aliquots of each pool were tested on two subsequent runs. Results were combined with the results from within-run precision (total of 20 replicates for each pool). The SD for low and high pools fall within manufacturer's claim of 0.10 $\log_{10}$ cp/mL (see Table 1).

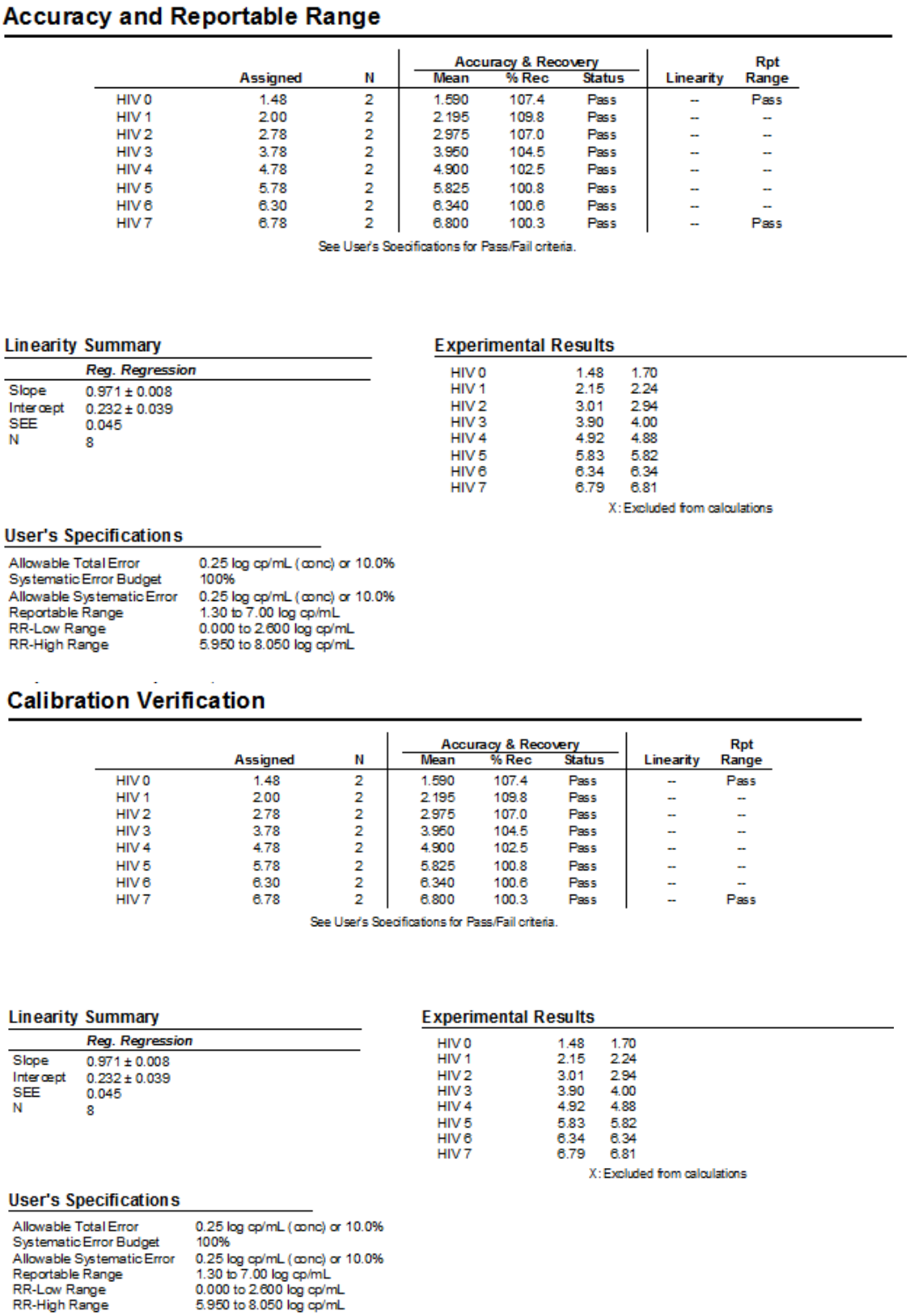
Table 1: Precision Summary

	Within Run (log10)				Between Run (log10)			
	Sample Number	Mean (log10)	SD	CV	Sample Number	Mean (log10)	SD	CV
Low Pool	10	3.62	0.068	1.87%	20	3.61	0.068	1.88%
High Pool	10	6.06	0.042	0.68%	20	6.06	0.054	0.90%

Accuracy, Reportable Range, AMR, and Linearity Verification Studies:

The purpose of these studies was to verify that the performance of the **cobas®** HIV-1 assay is capable of producing linear and accurate results spanning the assay's analytical measurement range. To accomplish this, two bottles each of seven HIV panel specimens were individually pooled. The results of each pool ranged from 2.00-6.78 $\log_{10}$ cp/mL. To reach the low end of the analytical measurement range, an additional sample was prepared by diluting the remaining pooled level 1 panel specimen with tested negative filler plasma. All aliquots were tested on a single run. Results are shown in Figure 1 below. Results passed calibration verification, accuracy, linearity, and reportable range passed across the full range tested.

Figure 1: Accuracy, Reportable Range, AMR, and Linearity Studies Summary



Accuracy and Reportable Range

	Assigned	N	Accuracy & Recovery Mean	% Rec	Status	Linearity	Rpt Range
HIV 0	1.48	2	1.590	107.4	Pass	--	Pass
HIV 1	2.00	2	2.195	109.8	Pass	--	--
HIV 2	2.78	2	2.975	107.0	Pass	--	--
HIV 3	3.78	2	3.950	104.5	Pass	--	--
HIV 4	4.78	2	4.900	102.5	Pass	--	--
HIV 5	5.78	2	5.825	100.8	Pass	--	--
HIV 6	6.30	2	6.340	100.6	Pass	--	--
HIV 7	6.78	2	6.800	100.3	Pass	--	Pass

See User's Specifications for Pass/Fail criteria.

Linearity Summary

	Reg. Regression
Slope	0.971 ± 0.008
Intercept	0.232 ± 0.039
SEE	0.045
N	8

Experimental Results

HIV 0	1.48	1.70
HIV 1	2.15	2.24
HIV 2	3.01	2.94
HIV 3	3.90	4.00
HIV 4	4.92	4.88
HIV 5	5.83	5.82
HIV 6	6.34	6.34
HIV 7	6.79	6.81

X: Excluded from calculations

User's Specifications

Allowable Total Error	0.25 log cp/mL (conc) or 10.0%
Systematic Error Budget	100%
Allowable Systematic Error	0.25 log cp/mL (conc) or 10.0%
Reportable Range	1.30 to 7.00 log cp/mL
RR-Low Range	0.000 to 2.600 log cp/mL
RR-High Range	5.950 to 8.050 log cp/mL

Calibration Verification

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HIV 3	3.78	2	3.950	104.5	Pass	--	--
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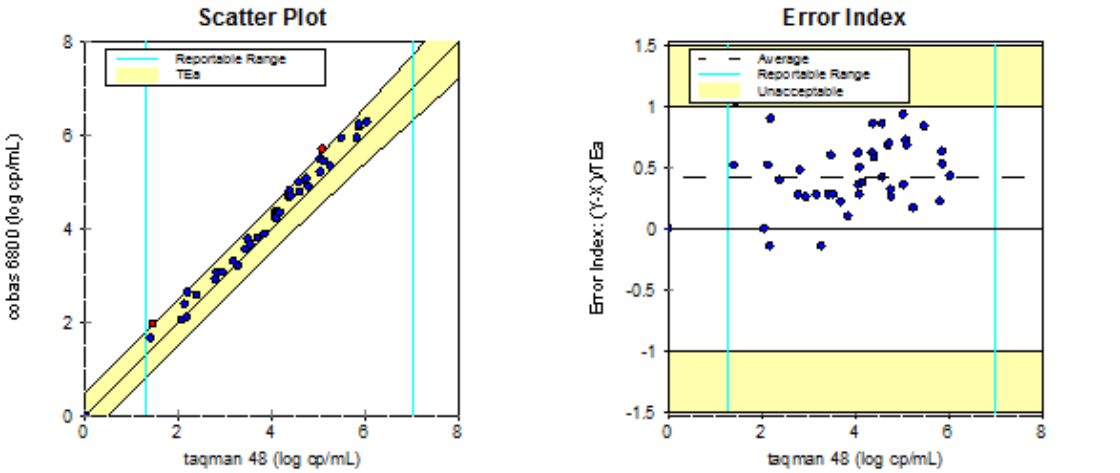
Method Comparison:

The purpose of this study was to verify that the performance of the HIV Viral Load assay on the **cobas**®6800 System is capable of providing comparable results when specimens previously tested on the **cobas®** Ampliprep/TaqMan Systems are re-assayed on the **cobas®** 6800 System. Fifty-four specimens were compared over a range of 0.00-6.78 $\log_{10}$ cp/mL. For the Two Instrument Comparison module (see Figure 2), two outliers were excluded, and EP Evaluator excluded two samples with acceptable results outside the reportable range, as it is unable to perform statistics on < or > values. Results passed within allowable error limits. The Alternate Method Comparison excluded no outliers and yielded an acceptable correlation factor. (see Figure 3).

Figure 2: Method Comparison: cobas® Ampliprep/Taqman System vs. cobas® 6800 (Two Instrument Comparison)

Two Instrument Comparison

X Method taqman 48 **Y Method cobas 6800**



Evaluation of Results

HIV VL was analyzed by methods taqman 48 and cobas 6800 to determine whether the methods are equivalent within AllowableTotal Error of 0.50 log cp/mL (conc) or 10%. 50 specimens were compared over a range of 0.00 to 6.02 log cp/mL. The test Passed. The difference between the two methods was within allowable error for 48 of 50 specimens (96.0%). The average Error Index (Y-X)/TEa was 0.41, with a range of -0.14 to 1.24. The largest Error Index occured at a concentration of 5.07 log cp/mL.

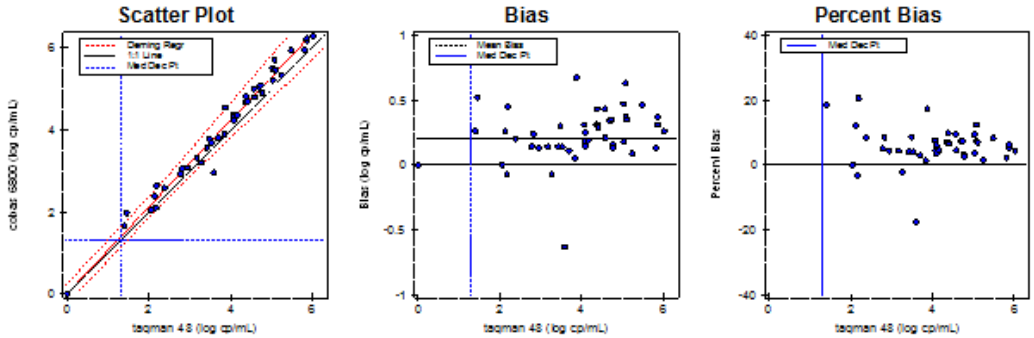
Key Statistics	
Average Error Index	0.41
Error Index Range	-0.14 to 1.24
Coverage Ratio	83%
Evaluation Criteria	
Allowable Total Error	0.50 log cp/mL (conc) or 10%
Reportable Range	1.30 to 7.00 log cp/mL

Deming Regression Statistics	
Y = Slope * X + Intercept	
Correlation Coeff (R)	0.9973
Slope	1.053 (1.031 to 1.075)
Intercept	0.030 (-0.057 to 0.116)
Std Error Estimate	0.140
N	50 of 54

Figure 3: Method Comparison: cobas® Ampliprep/Taqman System vs. cobas® 6800 (Alternate Method Comparison)

Alternate (Quantitative) Method Comparison

X Method taqman 48 **Y Method cobas 6800**



Regression Analysis

	Deming	Regular
Slope	1.056 (1.026 to 1.087)	1.050 (1.020 to 1.081)
Intercept	0.010 (-0.108 to 0.129)	0.030 (-0.088 to 0.149)
Std Err Est	0.192	0.192

95% Confidence Intervals are shown in parentheses

Medical Decision Point Analysis

Calculated by Deming Regression (R>=0.9)

X Method MDP	Y Method Pred. MDP	95% Conf. Limits Low	High
1.30	1.383	1.299	1.468

Supporting Statistics

Corr Coef (R)	0.9948	Std Dev Diffs	0.210	Scatter Plot Bounds	Allowable Total Error
Bias	0.204 (5.911 %)	SubRange Bounds	None		0.250 log cp/mL (conc) or 10.0%
X Mean ± SD	3.458 ± 1.771	Points (Plotted/Total)	52/54		
Y Mean ± SD	3.663 ± 1.870	Outliers	Not Tested		

Limitations:

1. This test is intended for use in conjunction with clinical presentation and other laboratory markers for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.
2. **cobas®** HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
3. **cobas®** HIV-1 has been evaluated only for use in combination with the **cobas®** HBV/HCV/HIV-1 Control Kit, **cobas®** NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
4. Reliable results depend on proper sample collection, storage and handling procedures.
5. Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods.
6. Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
7. The detection rate of HIV-1 group O at 20 cp/mL (claimed LLoQ for the **cobas**® HIV- 1) was observed to be 90.5% which is less than what was determined for all other genotypes.
8. Samples from subjects under 19 years of age were not evaluated.

References:

1. **cobas®** HIV-1 Package Insert, Version 1.0, January 2016