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

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HCV Real Time PCR Viral Load Assay

Contact:
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Effective Date:
April 1, 2009
Mnemonic:
HCVPCR
Performed:
Tuesday and Thursday (days)
Specimen:
2mL K2/K3 EDTA Plasma Frozen Aliquot
(Separate plasma within 6 hours, store 2-8C, freeze @ -70C within 72hr)

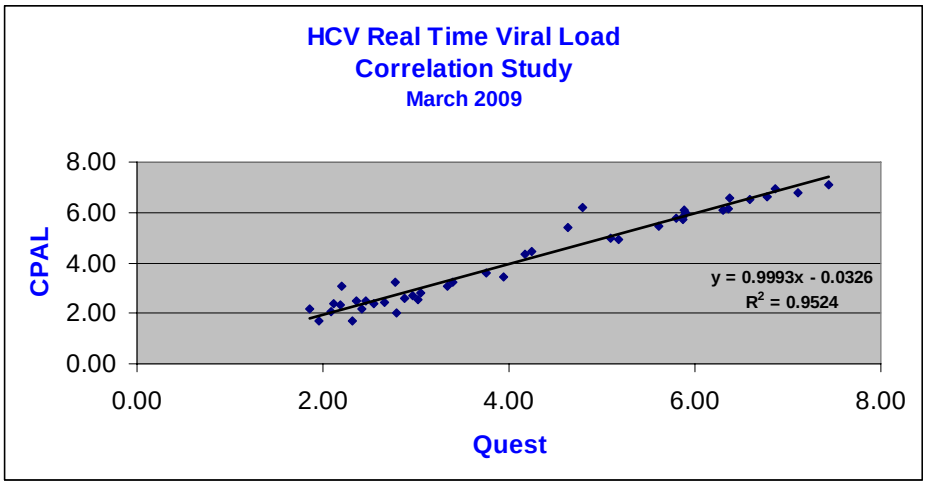
Summary:
Effective April 1, 2009 HCV Viral Load testing will be performed at CPAL using Real-Time quantitative PCR. The test method is the Roche Molecular Systems, Inc. *COBAS® AmpliPrep/COBAS® TaqMan® HCV Test.* CPAL has installed and verified the performance characteristics of the assay. This assay can quantitate HCV RNA over the range of **43 to 6,900,000,000 IU/mL** (**1.63 Log_{10} to 9.83 Log_{10}** HCV RNA IU/mL). ***See Note #1, below***.

Verification studies included analysis of assay performance for 53 comparative specimens obtained from our reference laboratory (Quest) and extensive studies using the OptiQuant® HCV RNA Quantification Panel (Acrometrix, Inc, Benicia, CA). The expected HCV viral loads of the panel members ranged from 50 IU/mL to 5,000,000 IU/mL.

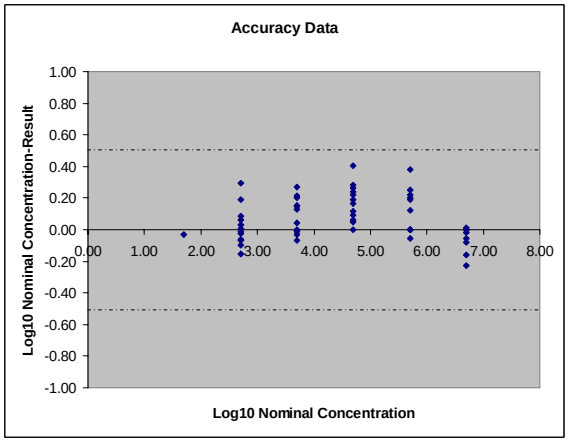
Regression analysis for 42 comparative specimens for which viral load data was generated is presented below. Correlation studies performed at CPAL verified that the assay performs as expected. An R^2 correlation coefficient of 0.9524 was obtained over a range of specimens with viral loads of 1.67 to 7.11 Log_{10} HCV RNA IU/mL. The slope of the regression line is

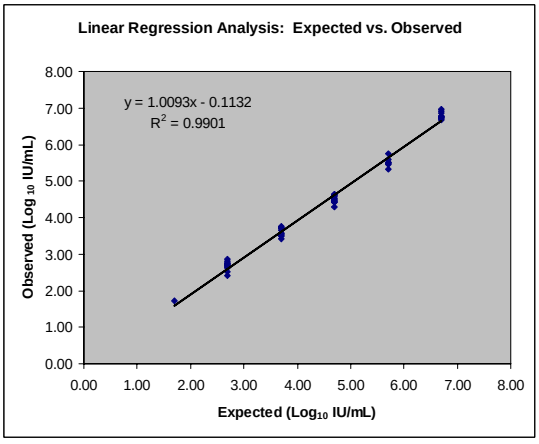
0.9993 (see below). The assay demonstrated good precision at all levels of the OptiQuant® HCV RNA Quantification Panel (All levels of the panel were run in quadruplicate).

Comparative Specimens



OptiQuant® HCV RNA Quantification Panel (Acrometrix, Inc, Benicia, CA)





Eleven specimens were included in the verification studies that were of low viral load. Seven specimens were determined by the reference laboratory to have viral loads of <50 IU/mL. Six of these seven generated viral loads of <43 IU/mL in our laboratory with one specimen generating a low result of 326 IU/mL. Four specimens that were reported by the reference laboratory to have low viral loads (81, 94, 118 and 136 IU/mL) were determined to have viral loads of <43 IU/mL in our laboratory. The OptiQuant® HCV RNA Quantification Panel contained a negative panel member in which no HCV is expected to be detected. This panel member was tested 16 times on fours separate occasions. On each occasion no HCV was detected.

Notes:

1. If another assay method (i.e. bDNA) was initially used for quantitation of HCV viral load RNA in order to assess treatment effect on a particular patient, it is important to note that significant differences exist in the quantitative values generated by different laboratory HCV viral load assays. It is recommended that, prior to switching to the *COBAS® AmpliPrep/COBAS® TaqMan® HCV Test*; a correlation is performed between methods.
2. Quantitation of HCV RNA is dependant on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (e.g. age, presence of symptoms) and stage of infection.
3. The *COBAS® AmpliPrep/COBAS® TaqMan® HCV Test* is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection.
4. Though rare, mutations in the highly conserved regions of the viral genome covered by the *COBAS® AmpliPrep/COBAS® TaqMan® HCV Test* primers and/or probe may result in the under-quantitation of or failure to detect the presence of the virus in this circumstance.
5. Results will be reported as HCV RNA IU/mL and Log_{10} IU/mL.