

Evaluation of the Roche COBAS Ampliprep/COBAS TaqMan HCV Test

Introduction

The COBAS Ampliprep/COBAS TaqMan HCV Test is an in-vitro nucleic acid amplification test for the quantification of Hepatitis C virus (HCV) RNA in human plasma or serum using the COBAS Ampliprep instrument for automated specimen processing and the COBAS TaqMan analyser or the COBAS TaqMan 48 analyser for automated amplification and detection. This assay was evaluated by the NRL as a reference test to be used for clinical monitoring and management.

Methods

Sensitivity

Sensitivity was estimated by testing 75 samples confirmed positive for HCV RNA. The samples tested were of genotypes one to six.

Linearity

Linearity was estimated by testing a number of replicates of each member of a nine-member dilution series. Ten replicates of six dilutions and 37 replicates of three dilutions were tested. The stock material of the dilution panel (a high titre HCV RNA positive clinical sample – genotype 5a) was quantified in the COBAS Ampliprep/COBAS TaqMan HCV Test prior to panel construction. The panel members were of half \log_{10} difference, ranging from 2.0 – 6.0 \log_{10} IU/mL. Regression analysis (correlation coefficient (r^2)) was used to estimate linearity.

Reproducibility

Three members of the linearity panel were tested at a greater frequency to enable an estimation of reproducibility at different intervals within the assay's quantification range. The members that were tested contained HCV RNA at a concentration of approximately at 5.5, 4.0 and 2.5 \log_{10} IU/mL. Additionally, a QC sample having a concentration of HCV RNA of approximately 4.25 \log_{10} IU/mL was tested in each assay run. The mean, standard deviation (SD) and coefficient of variation (CV) was calculated for the results obtained to establish the inter-run assay variation. All data from all replicates of each QC sample were analysed using Grubbs' test for the identification of outliers.

Results

Sensitivity

Of the 75 HCV RNA positive samples tested in this evaluation:

- Valid results were obtained for 67 samples. The results for 65 of these samples were within the assay's quantification range. HCV RNA was not detected in two samples.
 - The two samples in which HCV RNA was not detected had produced positive results when tested previously in the NRL's in-house qualitative HCV RT-PCR assay.
- HCV genotypes 1-6 were represented in the 67 samples that produced valid results.
- Two samples were not processed by the Ampliprep system because of an error code associated with insufficient sample volume.
- Invalid results (caused by invalid quantification standard results) were obtained for five samples.
 - These five samples had produced valid results when tested previously in other qualitative or quantitative HCV RNA assays.
- One sample's volume was lost because of a malfunction of the Ampliprep instrument.

Linearity

Of the 171 members of the dilution panel tested in this evaluation:

- Valid results were obtained for 161 members. The results for 159 members were within the assay's quantification range. HCV RNA was not detected in two members.
 - The two members in which HCV RNA was not detected were expected to have a concentration of HCV RNA of approximately 2.5log₁₀ IU/mL. A further 34 replicates of the same panel member were tested. All 34 of these replicates produced valid, quantified results.
- Three samples were not processed by the Ampliprep system because of an error code associated with insufficient sample volume.
- Four samples were not processed by the Ampliprep system because of an error code associated with the detection of a clot in each sample.
- Invalid results (caused by invalid quantification standard results) were obtained for two samples.
- One sample's volume was lost because of a malfunction of the Ampliprep instrument.

The correlation coefficient that was calculated from the valid results was estimated to be 0.99. This suggests that the assay produces linear results between 2.0log₁₀ IU/mL and 6.0log₁₀ IU/mL, as shown in Figure 1.

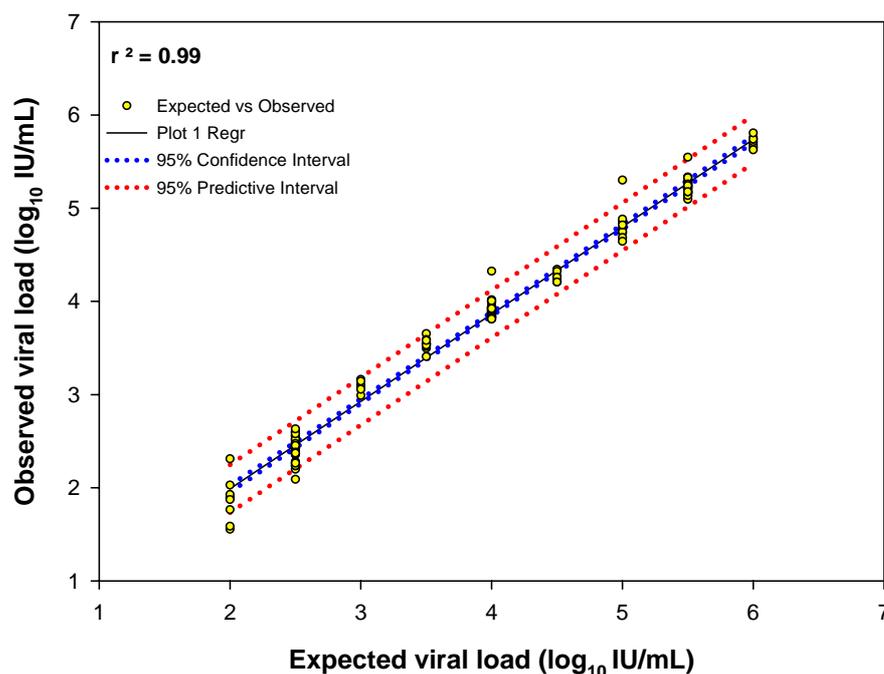


Figure 1: The linearity, 95% confidence interval and 95% predictive interval estimated from the results obtained by testing multiple replicates of a dilution series in the COBAS Ampliprep/COBAS TaqMan HCV Test. The members of the dilution series were of half log difference ranging from 2.0 – 6.0 log₁₀ IU/mL.

Reproducibility

The CV of results from four samples tested between 12 and 35 times ranged from 0.8 - 5.2%. The results are presented in Table 1 and Figure 2:

Table 1: The viral load results obtained by testing multiple replicates of four samples having different concentrations in the COBAS Ampliprep/COBAS TaqMan HCV Test.

Expected Viral Load (Log ₁₀ IU/mL)	Dilution Series Replicates			QC Sample
	5.50	4.00	2.50	4.25
Observed (mean) Viral Load (Log ₁₀ IU/mL)	5.24	3.91	2.42	4.29
N	33	35	34	12
SD	0.08	0.09	0.13	0.04
CV%	1.60	2.33	5.22	0.82

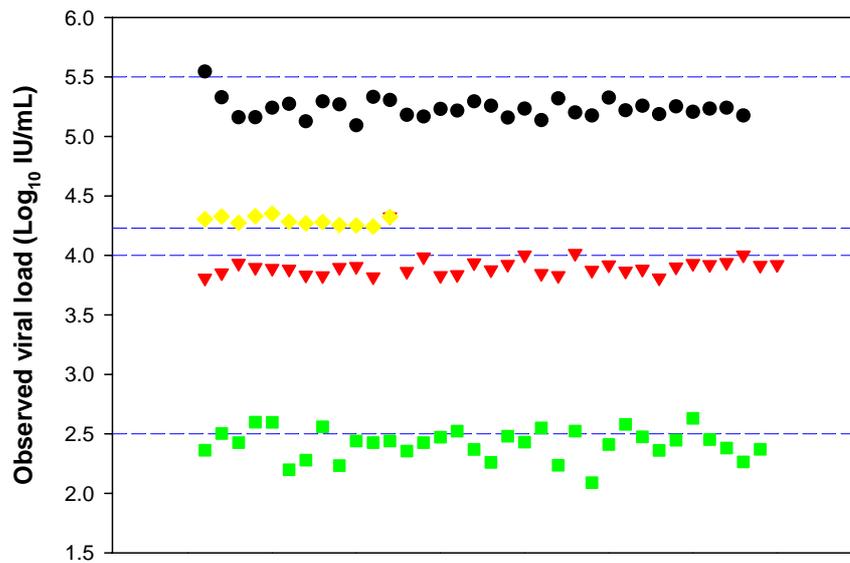


Figure 2: The precision of the viral load results obtained by testing multiple replicates of four samples having different concentrations in the COBAS Ampliprep/COBAS TaqMan HCV Test. The blue dashed lines represent the expected viral load result for each sample. The coloured symbols represent the results obtained for each sample.

The reproducibility was considered adequate for a NAT viral load assay.

This assay has since been registered on the Australian Register of Therapeutic Goods.

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