The AMPIPROBE™ HCV Assay Kit results presented here were evaluated for use with the QIAGEN® Qiagen RotorGene Q platform. The AMPIPROBE™ HCV Assay is a real-time reverse transcription quantitative polymerase chain reaction assay that incorporates probe detection technology in primer design. It is intended for the quantitative detection of HCV RNA, genotypes 1 through 6, in plasma or serum.

A quantitative HCV viral load assay using novel probe technology was validated for use in the quantitative detection of HCV RNA. The AMPIPROBE™ HCV Assay is a real-time reverse transcription quantitative polymerase chain reaction assay that incorporates probe detection technology in primer design. It is intended for the quantitative detection of HCV RNA, genotypes 1 through 6, in plasma or serum.

The limit of detection (LOD) of the assay was determined using spiked plasmad or serum specimens with Acrometrix® reference materials calibrated by using the World Health Organization HCV RNA standard in guidelines described in CLSI EP17-A. The LOD of HCV RNA in EDTA plasma was determined to be as low as 0.90 Log IU/mL and in serum as low as 0.74 Log IU/mL, both with a positive rate greater than 95%. For both plasma and serum, the lower limit of quantification was 1.0 Log IU/mL using a 95% hit rate analysis. A reference panel was utilized to confirm that the AMPIPROBE™ HCV Assay was able to detect genotypes 1a, 1b, 2a, 3a, 4acd, 5a, and 6 at 15 IU/mL or greater at a 95% hit rate. The linear range was determined to be from 0.7 to 7.4 Log IU/mL (5 to 25,000,000 IU/mL).

The LOD and LOD determined for the AMPIPROBE™ HCV Assay are lower than that of comparable HCV viral load assays, making it a more sensitive assay suitable for monitoring viral load during antiviral therapy.

**AMPIPROBE™ HCV ASSAY VALIDATION**
- The AMPIPROBE™ HCV Assay Kit results presented here were evaluated for use with the QIAGEN® QIAsymphony® SP and RotorGene® Q systems. All validation studies were performed with an input volume of 500 µl of either EDTA plasma or serum (600 µl on board volume).
- The validation package is currently undergoing review in the New York State Department of Health's comprehensive test approval process.
- The AcroMetrix® HCV-5 panel was used for the creation of all test sample dilutions. To verify the accuracy of the panel's calibrated values it was verified with the 4th World Health Organization HCV viral load reference panel.
- For genotype inclusivity, the AMPIPROBE™ HCV Assay detected the seven different genotypes/subtypes at least 95% hit rate across all genotypes.

**ENZO’S AMPIPROBE™ TECHNOLOGY**
- Real-time reverse transcription quantitative PCR
- Incorporates probe detection technology in primer design
- Fluorescent reporter-labeled primers
  - Quencher-labeled primers
- When free in solution, fluorescent primers generate a signal. However, as the primers are incorporated into amplified DNA, the quencher and the fluorophore are brought within close proximity and exhibit Förster resonance energy transfer (FRET).
- Enzo’s AMPIPROBE™ Assay Kits provide the following benefits:
  - Compatible with open qPCR platforms
  - Smaller sample input allows remaining extracted samples to be used in other tests
  - Smaller reaction volume consumes less reagents

**SENSITIVITY – LIMIT OF DETECTION**
To determine plasma or serum LOD, seven dilutions of spiked plasma samples with low concentrations (6.00 to 1.00 Log IU/mL of HCV RNA were prepared by spiking known quantity of HCV RNA (Acrometrix HCV-5A) into HCV-negative plasma specimens. For plasma, nine separate extractions and PCR runs were performed on nine different days using three different reagent lots. For serum, three separate extractions and PCR runs were performed on three different days using three different reagent lots. Each replicate of each dilution level were run each day, resulting in 18 replicate data points for each dilution. For serum, separate extractions and PCR runs were performed on three different days using three different reagent lots. Each replicate of each dilution level were run each day, resulting in 6-9 replicate data points for each dilution.

**GENOTYPE INCLUSIVITY**
Genotype verification of the LOD was performed using one reference sample member from the SeraCare HCV Worldwide Accuset® Performance Panel for each different-genotype/subtype represented in the panel (1a, 1b, 2a, 3a, 4acd, 5a, 6b) for a total of seven different genotypes/subtypes. Each panel member was used to create plasma spiked in samples at 3 different concentrations (15, 45, and 450 IU/mL). Each concentration for all genotypes was performed in duplicate across 3 days using 3 different lots of all reagents.

**LINEAR RANGE**
The linearity of plasma and serum was analyzed using the CSU StatPro™. The AMPIPROBE™ HCV Assay demonstrated a linear response from 0.7 to 7.4 Log IU/mL (5 to 25,000,000 IU/mL).

**CONCLUSIONS**
A sensitive, real-time reverse transcription polymerase chain reaction assay is recommended to detect HCV RNA levels during treatment with direct-acting antiviral agents. Compared to HCV viral load assays available, the LOD and LOD determined for the AMPIPROBE™ HCV Assay are lower, making it a more sensitive assay suitable for monitoring viral load during antiviral therapy.