Hepatitis C
Requirements of Antiviral Therapy Monitoring

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Nucleic acid amplification tests (NAATs) based on PCR are currently regarded as the gold standard for the development diagnosis of both acute and chronic hepatitis C (HCV) infections. In order to monitor the therapeutic effectiveness and recovery process, a highly sensitive quantitative HCV RNA assay with a detection limit in the range of 12 to 25 IU/ml is required. In addition, a precise quantification of the viral load of high-to-weak positive concentration ranges of HCV RNA during the entire therapeutic process is vital for treatment to be successful.

The hepatitis C virus – a short profile

The hepatitis C virus was identified for the first time in 1989 with the help of genetic engineering methods. It is an enveloped single(+)-stranded RNA virus. Currently seven genotypes and 86 subtypes are known. According to the World Health Organization, 130 to 150 million people are infected with hepatitis C, representing 2 to 2.5 percent of the world population. Furthermore, there are substantial regional differences in terms of the virus' prevalence. For example, prevalence exceeds 10% of the population in Egypt, while only 0.3% of the population in Germany is affected. However, hepatitis C infection is the most common cause of liver cell carcinoma and liver transplants in Europe, which are possible late-stage effects of chronic infection alongside cirrhosis of the liver. In Europe, the USA and Asia, genotypes 1, 2, and 3 are primarily seen, while in Africa, genotype 4 is by far the most common. The human-specific virus is mainly transmitted by blood. Therefore, intravenous drug users who use contaminated syringes are most commonly affected. Homosexual men and dialysis patients are regarded as additional risk groups.

The hepatitis C infection is nowadays curable.

The objective of treatment is to cure the infection by eliminating the hepatitis C virus. HCV RNA in the blood is regarded as a surrogate marker. The treatment process is evaluated using the *sustained viral response* (SVR). For this, a sensitive molecular biology assay is used during treatment after 2 (SVR2), 12 (SVR12), and/or 24 weeks (SVR24), as well as after treatment has ended, according to the guidelines of the *European Association for the Study of the Liver* (EASL). Until 2011, the combination of pegylated interferon (PegIFN)-α and ribavirin for 24 or 48 weeks was the standard treatment for hepatitis C. Depending on the respective genotype, however, only insufficient SVR rates of 40–80% were achieved. Since 2011, a series of direct antiviral agents (DAAs) was approved, offering the possibility of a combination therapy that is highly effective, has few side effects and is interferon-free as an alternative for patients with a chronic HCV infection. Studies have shown SVR rates of more than 90% [1-3].

Testing for HCV involves a continuum.

The detection of the HCV infection generally begins with serological tests to detect anti-HCV antibodies. However, antibody tests for HCV are not perfect. A positive antibody test means that the person in question had contact with the virus in the past but does not necessarily mean that the individual is a carrier of an active HCV infection, since the individual may have spontaneously cleared the infection on his or her own. Alternatively, a person who has been recently infected with HCV may not have produced any HCV antibodies yet. Consequently the result remains negative. According to estimates, approximately 25% of people who were initially infected with HCV are able to clear the infection on their own within 6 months, while the remaining individuals develop chronic hepatitis C. Approximately 20–30% of patients with persistent viremia develop liver fibrosis and cirrhosis, liver failure and liver cell carcinoma. Therefore, all individuals with positive results from anti-HCV screening assays should undergo an HCV confirmation test to determine whether positive serological results indicate the presence of an active infection [4].

Nucleic acid amplification tests (NAATs) based on PCR are currently regarded as the gold standard for the diagnosis of an HCV infection. The standardized approach for monitoring the effectiveness of treatment involves the repeated quantitative measurement of the patient's HCV RNA levels. Monitoring requires the use of a highly sensitive HCV RNA assay, typically with a detection limit of < 25 IU/ml. With the introduction of DAAs and the determination that single measurements are adequate for monitoring treatment [5], it is vital that the NAAT assays used for the treatment process provide precise quantification results and have a very sensitive detection limit. Analytik Jena has recently introduced an appropriate comprehensive solution for the precise and robust collection of clinically relevant data. For this, the extraction of viral RNA is performed with the INSTANT Virus RNA/DNA Kit, followed by quantification on the qTOWER³ using the RoboGene® HCV RNA Quantification Kit 3.0.

HCV Monitoring With the RoboGene® HCV RNA Quantification Kit 3.0 in qTOWER³

The data presented below from the clinical validation demonstrate the reliability of the RoboGene® HCV RNA Quantification Kit 3.0 with qTOWER³. The detection limit was determined using the dilution series of the PEI reference material for HCV RNA (#3443/04, genotype 1). For the subsequent PROBIT analysis (Figure 1), at least 29 replicats at each concentration were determined with 95% accuracy. A detection limit of 17 IU/ml was determined with the qTOWER³ for the real-time PCR-based detection of HCV RNA. Detection is possible below this detection limit but is subject to a greater margin of error. For the diagnostic evaluation of the RoboGene® HCV RNA Quantification Kit 3.0, a total of 100 patient samples were assessed. These samples had been previously evaluated as HCV positive via CAP/CTM (Roche). Aside from highly variable initial concentrations, these samples included various representative genotypes for the European region such as 1a, 1b, 2a, 2c, 3a, 4a, 4c and 4d. For the comparison, presented in Figure 2, an acceptable range of +/- 1 $\log_{10}$ was assumed. Taking into account statistical inaccuracies and the variability of individual datasets (Deming regression), no significant differences could be found between the two methods.
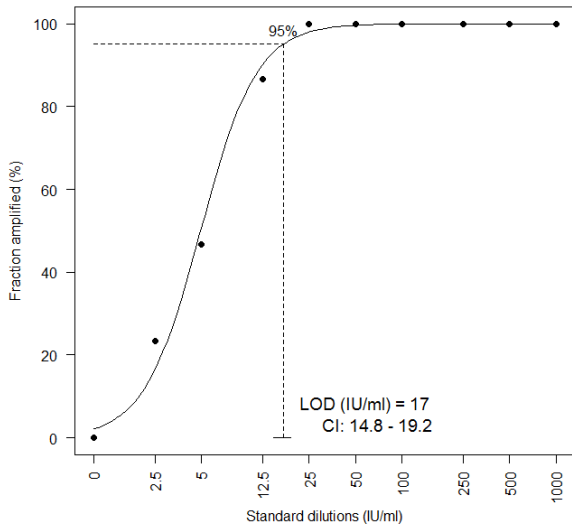
Fig. 1: Profit analysis of the determination of analytical sensitivity with 95% accuracy.
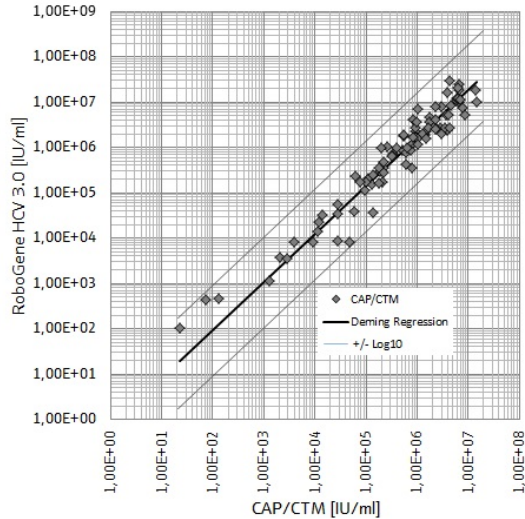

Fig. 2: Quantification results of the RoboGene® HCV RNA Quantification Kit 3.0 in correlation with the CAP/CTM assay (Roche).

In addition, a genotype panel of the University of Essen, including the genotypes 1a, 1b, 2a, 2b, 2c, 2i, 3a, 4a, 5a, and 6e (semilogarithmic dilution stages), was analyzed. The results of the RoboGene® HCV RNA Quantification Kit 3.0 were compared with results that were achieved with the Abbott RealTime HCV Assay within the $\pm \log_{10}$ acceptance interval. All of the tested subtypes demonstrated comparable quantification efficiencies, as represented in Figure 3.
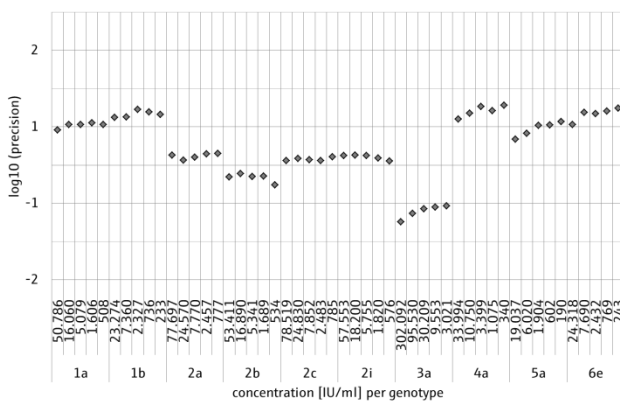

Fig. 3: Precision quantification analysis of the dilution series of various subtypes from the genotype panel of the University of Essen, compared with the results of the Abbott RealTime HCV Assay.

In order to determine the analytic precision of the RoboGene® HCV RNA Quantification Kit 3.0, three different viral loads were selected in the low viremic range and each was analyzed on three different real-time PCR platforms (qTOWER³, CFX96, and LightCycler 480). This was also done on three successive days, with three different kit charges respectively.

In order to consider all of the variables (day, real-time PCR cycler, charge), accuracy and variability were analyzed under constant measurement conditions (intra-assay variability), as well as the variability between measurement conditions (inter-assay variability). An overview of the specifications is shown in Table 1. As shown in Table 1, an intra-assay variability of 13-23% and an inter-assay variability of 20-33% were determined, confirming the performance of the RoboGene® HCV RNA Kit 3.0.

Table 1: Accuracy, inter- and intra-assay variability of the RoboGene® HCV RNA Quantification Kit 3.0

HCV RNA concentration [IU/ml] used	Mean HCV RNA concentration [IU/ml] detected	Accuracy	$\log_{10}$ Accuracy	Intra-assay precision		Inter-assay precision	
				SD [IU/ml]	CV [%]	SD [IU/ml]	CV [%]
25000	26303	1.05	0.02	3.870	15	5200	20
2500	2764	1.11	0.04	373	13	565	20
250	237	0.95	-0.02	55	23	79	33

The quantification standards contained in the kit are decisive here. Standard concentrations of 10^7, 10^6, 10^5, and 10^4 IU/ml are generally used for the products available on the market, covering the higher viral loads in human plasma. By contrast, the RoboGene® HCV RNA Kit 3.0 contains standards with concentrations of 4×10^7, 4×10^5, 4×10^3, and 4×10^2 IU/ml, covering the full clinically relevant range of viral loads, thereby enabling a particularly reliable quantification as part of monitoring treatment.

Reliable analysis over the entire period of HCV therapeutic monitoring

Since the introduction of treatment with DAAs, the detection limit of 25 IU/ml, coupled with high precision in the low viremic range, has assumed an even more important role for the evaluation of SVR according to procedure. In short, the studies shown here indicate that the CE IvD-certified workflow of the INSTANT Virus RNA/DNA Kit for nucleic acid purification, combined with the RoboGene® HCV RNA Quantification Kit 3.0 for nucleic acid detection on the Real-Time PCR Cycler qTOWER³, achieves outstanding results and can be used reliably over the entire period of HCV treatment monitoring.

References

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