MIKROGEN DIAGNOSTIK

Updated version

*ampli*Cube HEV 2.0 Quant

Real-time RT-PCR for detection and quantification of Hepatitis E virus (HEV) RNA in human plasma, serum or stool.

Hepatitis E virus is one of the most common viral causes of acute hepatitis worldwide. An infection can have an inapparent to fulminant clinical manifestation. The acute hepatitis E infection is comparable to that of a hepatitis A infection. It causes flu-like symptoms, nausea, vomiting / diarrhea, fever, joint and headaches and is associated by an increase in liver enzymes. A HEV infection is usually self-limiting.

There are four human pathogenic HEV genotypes (1–4) described that differ in their geographical distribution, transmission and possible complications.

The HEV genotypes 1 and 2 occur primarily in developing countries and the transmission is usually fecal-oral via contaminated drinking water. During pregnancy a high percentage of these HEV infections can have a fulminant progression with a high fatality rate (~20%). In industrial countries the HEV genotypes 3 and 4 are widespread and are usually transmitted by infected pork that has not been adequately cooked. Cases involving transmission by blood products or transplants have also been described. For this reason and due to the partly high seroprevalences described (up to 50%), some European countries have already included HEV in the universal blood donor screening using nucleic acid testing (NAT). Most cases in Europe are caused by HEV genotype 3 and are often asymptomatic. Immunosuppressed patients are a risk group in this regard because more than 50% of these patients develop chronic HEV disease and a rapid progression to liver cirrhosis is possible. Determination of the viral load using quantitative NAT-assays is necessary to monitor such patients and their therapy. Mainly in the context of HEV Genotype 3 infections, also extrahepatic manifestations have been described, such as neurological injuries like neuralgic amyotrophy (NA) or the Guillain-Barré syndrome (GBS).

The *ampli*Cube HEV 2.0 Quant is a highly sensitive and flexible real-time RT-PCR test system for the detection of the four human pathogenic HEV genotypes in human plasma, serum or stool. The test can deliver qualitative and/or quantitative results and its performance is suitable for blood donor screening.

Product Advantages for Your Benefit

- High sensitivity (LoD: 36.13 IU/ml), suitable for single sample testing (ID-NAT) and sample pool testing (MP-NAT)
- Detection of all four human pathogenic genotypes occurring worldwide
- Qualitative and/or quantitative evaluation, determination of viral load (IU/ml) possible
- Validated for various types of sample material (human plasma, serum and stool)
- Easy to use: enzyme mix is coloured blue for visual control
- Complete: positive and negative control, internal control (extraction and inhibition control) as well as standards for quantification
- High flexibility: use of different extraction methods and real-time PCR cycler possible
- CE label: The *ampli*Cube HEV 2.0 Quant test meets the high standards of the European Directive 98/79/EC on *in vitro* diagnostic medical devices

Testprinciple and Procedure

Sample	Nucleic acid extraction	PCR reaction	Amplification and detection
Plasma, serum or stool	30 minutes	30 µl master mix 20 µl eluate	90 minutes HEV: FAM (510 nm) IC: ATTO 647N (660 nm)

Evaluation

The *ampli*Cube HEV 2.0 Quant has been fully validated with LightCycler Instrument 480 II (Roche). For extraction of nucleic acid the MagNAPure Compact (Roche) and the Total Nucleic Acid Kit I (Roche) were used. (A compatibility list is available for additional extraction and detection methods.)

Sensitivity and Specificity

*ampli*Cube HEV 2.0 Quant	Defined samples	
	Positive* (n = 72)	Negative** (n = 75)
Positive	72	0
Negative	0	75
Sensitivity	100 %	
Specificity	100 %	

* HEV-positive plasma, serum and stool samples
** HEV-negative plasma, serum and stool samples

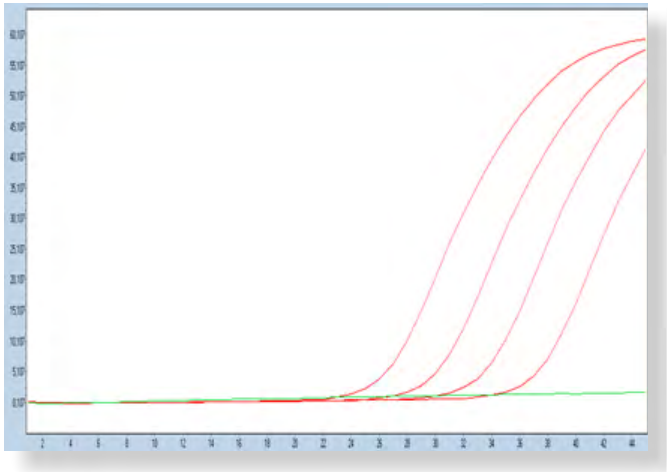
Sample	Limit of Detection (LoD)		
	Plasma	Serum	Stool
*ampli*Cube HEV 2.0 Quant	36.13 IU/ml (24.80 - 78.16)	< 50 IU/ml	< 100 IU/ml

In plasma the limit of detection (LoD) of the previous test version *ampli*Cube HEV 2.0 was determined to be 36.13 IU/mL (95% confidence interval: 24.80 - 78.16 IU/mL) using probit regression analysis. This LoD was confirmed for *ampli*Cube HEV 2.0 Quant by performing different comparison studies with the *ampli*Cube HEV 2.0.
The LoD for *ampli*Cube HEV 2.0 Quant in serum and stool samples was determined by analysing dilution series of the HEV standard in serum and stool in three replicates. The concentration of the lowest dilution step at which all replicates tested positive was defined as the LoD and was confirmed by the analysis of 20 independent samples with this concentration.

Detection of human pathogenic HEV genotypes

Sample	Genotype	Material	Origin	*ampli*Cube HEV 2.0 Quant	
				HEV	IC
8567	1a	plasma	India	positive	valide
8568	1a	stool	India	positive	valide
8569	1e	plasma	Sudan	positive	valide
8570	3b	plasma	Japan	positive	valide
8571	3c	plasma	Sweden	positive	valide
8572	3e	plasma	Germany	positive	valide
8573	3f	plasma	Sweden	positive	valide
8574	3 (rabbit)	stool	France	positive	valide
8575	4c	plasma	Japan	positive	valide
8576	4g	plasma	Japan	positive	valide
8576	2a	stool	Mexico	positive	valide

Specifications and analysis of the WHO HEV genotype panel („1st WHO International Reference Panel for Hepatitis E (HEV) Genotypes for Nucleic Acid Amplification Technique (NAT)-Based Assays“ PEI code 8578/13) with *ampli*Cube HEV 2.0 Quant.



Detection of the defined standards from *ampli*Cube HEV 2.0 Quant with concentrations of 10^1, 10^2, 10^3 und 10^4 IU/µl (red) and a negative HEV sample (green) in the FAM detection channel (510 nm).

Article No.

55002 ***ampli*Cube HEV 2.0 Quant**
Reagents for 50 determinations

Storage

At -25 °C to -18 °C

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