Validation of a New Highly Sensitive Real Time RT-PCR for Detection of Hepatitis E Virus in Human Plasma Pool Samples

T. Vollmer1, J. Dreier1, M. Sander2, O. Matsuki1, B. Krämer2, C. Reichhuber2, F. Schwarzmann2, E. Soutschek2, O. Böcher2
1 Institute for Laboratory and Transfusion Medicine, Bad Oeynhausen, Germany
2 Mikrogen GmbH, Neuried, Germany

Introduction and Purpose
During the past years multiple HEV infections without confirmed travel history (India, Pakistan, Mexico, South-East Asia) have been diagnosed as autochthonous HEV infections in the northern industrialized countries. Human Hepatitis E Virus (HEV) infections can present from inapparent to fulminant clinical pictures. In most cases HEV infections are acute, self-limiting viral hepatitis infections, comparable to Hepatitis A (HAV) infections. But recently individual chronic cases have been reported, particularly in immunocompromised patients. Parenteral transmission through blood products and organ transplantation is likely besides the usual fecal-oral transmission route. Human-pathogenic HEV is distributed worldwide in 4 different genotypes. The human genotype 3 shows high homology with swine HEV, which is currently under discussion. Based on unexpected high IgG prevalences against HEV in industrialized countries it is assumed that HEV infections often remain underdiagnosed. In this study the new highly sensitive real-time RT-PCR assay ampliCube HEV 2.0 has been validated at the Institute for Laboratory and Transfusion Medicine Bad Oeynhausen, Germany, with an RNA extraction protocol for single sample screening and another high volume protocol for pooling of blood donor minipool samples.

Materials and Methods
RNA extraction: single sample protocol
The NucliSense easyMag (bioMérieux, Nürtlingen, Germany) was performed for extraction of total RNA from 500µl plasma. RNA was eluted in 55µl elution buffer.

RNA extraction: high volume protocol for pool samples
The chemagic viral RNA/DNA virus kit, 4.8 ml Protocol (Perkin Elmer/Chemagen, Baesweiler, Germany) was performed for extraction of RNA from pool samples in combination with the automated chemagic MS instrument magnetic separation module (Perkin Elmer/Chemagen, Baesweiler, Germany). RNA was eluted in 100µl elution buffer.

RT-PCR assay and instrument
For the detection of viral nucleic acid (HEV-RNA) in EDTA- and Citrat-Plasma by Reverse Transcription and Polymerase Chain Reaction (RT-PCR) the ampliCube HEV 2.0 assay (MIKROGEN GmbH, Neuried, Germany) and Rotorgene3000/Q Real-Time PCR platform (Qiagen Tidenhilden Germany) has been used. The assay has been approved according to IVD guideline. The method has been developed by the manufacturer and has been validated in combination with two different extraction assays: the chemagic viral RNA/DNA virus kit, 4.8 ml Protocol (Perkin Elmer/Chemagen) and the NucliSense easyMag kit in combination with extraction instrument NucliSense easyMag (bioMérieux). The ampliCube HEV 2.0 assay is a ready-to-use reagent system for the detection of HEV-RNA by Real-Time PCR using the Rotorgene3000/Q. The assay has all required reagents and enzymes included in the kit for reverse transcription and specific amplification of the target region of the HEV genome as well as for direct detection of an amplicon in the FAM channel (green) of Rotorgene3000/Q. Additionally the primer and probe mix of the assay contains a further amplification system for control of possible PCR inhibition in case of missing amplification of HEV-RNA, which is detected as internal reaction control in CY5 channel (red) and does not interfere with the analytical HEV-RT-PCR.

Analytical Sensitivity and Precision
Two-fold dilution series of plasma samples inoculated with the 1st WHO International Standard for HEV-RNA (WHO-NAT Standard, Paul-Ehrlich-Institute, Langen, Germany) was used for determination of the analytical sensitivity and intra-/inter-assay variation of the ampliCube HEV 2.0 (Table 1).

The 95% Limit of Detection (LOD) was calculated by PROBIT analysis with 6 dilution steps using SPSS software (SPSS GmbH Software, version 14, Munich, Germany) for both RNA extraction protocols. The reproducibility of the ampliCube HEV 2.0 was demonstrated by analyzing the intra-/inter-assay variation for Ct (cycle threshold) value. The intra-assay variability was determined from three independent PCR runs with four replicates per run (Table 2).

Conclusions
The new ampliCube HEV 2.0 assay is suited perfectly for screening of individuals for HEV infection. The sensitivity of the assay allows a pool screening strategy for blood donor screening. The assay provided a excellent analytical sensitivity with high reproducibility for both NAT-extraction methods.