Evaluation of the sensitivity of RealStar® HEV RT-PCR Kit 2.0 based on three commonly used extraction methods

Background and Aim

Hepatitis E virus (HEV) is a small, non-enveloped, single-stranded RNA virus that causes hepatitis worldwide. Clinical and epidemiological presentation of hepatitis E varies greatly by location and is affected by the HEV genotype ([1] Baylis et al.). Of four major HEV genotypes which infect humans, genotypes 1 and 2 are endemic and responsible for waterborne epidemics, whereas genotypes 3 and 4 are originating from animal reservoirs ([2] Domanović et al.).

A substantial increase in acquired HEV cases is observed across Europe where HEV genotype 3 infections are predominant and have become a common cause of acute viral hepatitis ([3] Boland et al.). Nucleic acid amplification technique (NAT)-based assays are important for the detection of acute HEV infection as well for monitoring chronic cases of hepatitis E ([1]).

The Paul-Ehrlich-Institute (PEI, Federal Institute for Vaccines and Biomedicines), in Germany responsible for implementation and coordination of necessary measures for preventing public and animal health hazards, requires testing of blood components for transfusion, stem cell preparations from cord blood and for haematopoietic reconstitutions for HEV using a suitable procedure including the extraction and amplification of nucleic acids. An important step in the molecular detection of viruses in clinical specimens is the efficient extraction of viral nucleic acids. The total yield of viral nucleic acid from a clinical specimen is dependent on the specimen’s volume, the initial virus concentration and the effectiveness provided by the extraction method.

Based on the 1st WHO International Standard for Hepatitis E Virus (genotype 3a (PEI code: 6329/10)) at least 2000 IU/ml of single donor sample need to be detected reliably by a CE-marked NAT assay. The aim of this study is to show the high sensitivity of the RealStar® HEV RT-PCR Kit 2.0 for HEV genotypes 1 to 4 in combination with three commonly used extraction systems.

Method

Three commonly used and in many labs established extractions methods were compared to show their suitability for sensitive detection of HEV in combination with RealStar® HEV RT-PCR Kit 2.0: QIAamp Viral RNA Kit (manual extraction), automated NUCLISENS® EASYMAG® and the MagNA Pure 96 extraction system.

For the comparison serial dilutions of the 1st WHO International Standard for Hepatitis E Virus (genotype 3a (PEI code: 6329/10)) were used. All PCRs were performed on the real-time PCR cycler CFX96TM Deep Well Real-Time PCR Detection System (Bio-Rad).

Results

Depending on the extraction method, the LoD of the RealStar® HEV RT-PCR Kit 2.0 was 32 IU/ml to 50 IU/ml. The LoDs were confirmed for all genotypes (Table 2: Limit of Detection (LoD) and Genotype (GT) confirmation for tested workflows).

Table 1: Workflows for evaluation study

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Real-time PCR Kit</th>
<th>Real-time PCR Cycler</th>
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<tbody>
<tr>
<td>QIAamp Viral RNA Kit (QIAGEN)</td>
<td>RealStar® HEV RT-PCR Kit 2.0</td>
<td>CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)</td>
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<td>NUCLISENS® EASYMAG® (BioMérieux)</td>
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<td>MagNA Pure 96 Instrument (Roche)</td>
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The limit of detection for each workflow was calculated individually using Probit analysis (StatsDirect Statistical Analysis Software (StatsDirect Ltd, UK)). According to “Anforderungen an die Validierung bzw. den Routinetrieb von Nukleinsäure-Amplifikations-Techniken (NATs) zum Nachweis von Virusnukleinsäuren in Spenderblut” (PEI) the LoD (Limit of Detection) was confirmed for the genotypes 1, 2 and 4 (1st WHO International Reference Panel for Hepatitis E Virus Genotypes (PEI code: 8578/13)).

The trueness of the results generated using the RealStar® HEV RT-PCR Kit 2.0 was evaluated by testing several replicates of two dilutions (50,000 IU/ml and 5,000 IU/ml) of the 1st WHO International Standard (PEI code: 6329/10) within the linear range of the kit.

Conclusion

The RealStar® HEV RT-PCR Kit 2.0 allows sensitive detection and reliable quantification of HEV RNA in human EDTA plasma, independent of the extraction methods and real-time PCR cyclers used.

References


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