Performance of two commercial assays for detecting HEV RNA in acute or chronic infections

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Abstract

We assessed the performance of the Ceeram and Altona assays, the first two commercially available HEV RNA assays, using serial dilution of 4 HEV-positive reference samples (genotypes 3a, 3c, 3e, 3f). Both assays provided good analytical sensitivity and high reproducibility for detecting genotype 3 HEV RNA.
Hepatitis E virus (HEV) is becoming increasingly important in industrialized countries (1, 2). Four main genotypes and several subtypes have been identified (3). Most infections in industrialized countries are due to zoonotic transmission, often of genotype 3; subtypes 3a and 3b are frequent in North America and Japan and subtypes 3c, 3e and 3f are more prevalent in Europe (3-5). HEV3 is an emerging concern for immunocompromised patients, as it can lead to chronic infection and cirrhosis (6-12).

As evaluations of anti-HEV IgM assays revealed appreciable variations in their performances (13, 14), it is important to diagnose HEV infections by detecting HEV RNA. Several in-house RT-PCRs were recently evaluated and their sensitivities shown to differ greatly (15). We have also shown that genotype 3 diversity can influence the quantification of HEV RNA (16).

We have therefore assessed the performance of two newly available commercial HEV RNA assays, the Ceeram and Altona assays. We tested their ability to detect HEV RNA, particularly those subtypes of HEV3 that are most prevalent in industrialized countries.

We used the HEV RNA WHO international standard (WHO/BS/2011.2175), which is a HEV genotype 3a strain quantified at 250,000 IU/ml. Samples of HEV genotypes 3c, 3e and 3f were collected from patients in France (17, 18). Each sample was diluted in HEV-negative plasma and quantified with a validated in-house RT-PCR protocol using a transcribed RNA as quantification standard (1 IU/ml corresponds to 1.25 copies/ml) (16). HEV RNA was extracted from blood samples (140 µl) with the RNeasy Mini Kit according to the manufacturer’s instructions (Qiagen, Courtabeuf, France). The HepatitisE@ceeramTools kit by Ceeram (Ceeram S.A.S., La Chapelle sur Erdre, France)
France) and the RealStar® HEV RT-PCR Kit 1.0 by Altona Diagnostics (Eurobio, Courtaboeuf, France) were used with the Light Cycler 480 instrument (Roche Diagnostics, Meylan, France), according to the manufacturers’ instructions. The Ct (threshold cycle) value of each sample was determined.

The linearity of both assays was assessed with serial dilutions of the WHO HEV reference standard. The Ceeram assay was linear from 100 to 250,000 IU/ml and the Altona assay from 20 to 250,000 IU/ml (Figure 1). The standard curves gave amplification efficiencies of 2.08 for the Ceeram assay and 2.3 for the Altona assay. Reproducibility was estimated from the Ct values for each dilution. The mean standard deviation was 0.7 Ct (range: 0.4 - 1.6 log Ct) for the Ceeram RT-PCR and 0.4 Ct (range: 0.1 - 1.4 Ct) for the Altona RT-PCR.

We assayed samples of strains 3a, 3c, 3e and 3f to assess analytical sensitivity. The dilution concentrations were 2500, 500, 100 and 20 IU/ml and 6 replicates of each were assayed (Table 1). Both assays detected all the 2500 IU/ml and 500 IU/ml samples. The Ceeram assay detected 21/24 100 IU/ml samples, while the Altona assay detected all 24. The Ceeram assay detected 8/24 of the lowest concentration (20 IU/ml) samples, while the Altona assay detected 18/24 (p=0.008) (Table 1). The poorer sensitivity of the Ceeram assay at this low HEV RNA concentration was independent of a particular genotype 3 subtype. The Ceeram assay gave higher Ct values than the Altona assay (p=0.003). The mean difference in the Ct values was 3.4 Ct. The difference was 2.9 Ct for subtype 3a, 2.8 Ct for genotype 3c, 5.2 Ct for genotype 3e and 2.7 Ct for subtype 3f. The mean Ct difference was greater for subtypes 3e than for the other
subtypes (p<0.01). The Ceeram and the Altona RT-PCR results were correlated 
(ρ=0.88, p<0.001) (Figure 2).

A recent evaluation of homebrew HEV RNA assays using 10-fold serial dilutions 
of HEV reference samples (3a, 3b, 3f and 4c) found an enormous difference in their 
sensitivities (100-fold to 1,000-fold) (15). We therefore estimated the analytical 
sensitivity of commercial assays by testing serial dilutions of genotypes 3a, 3c, 3e, and 
3f reference strains. It was between 100 and 500 IU/ml for the Ceeram assay and 
between 20 and 100 IU/ml for the Altona assay. The Ceeram RT-PCR was less 
sensitive than the Altona RT-PCR when the HEV RNA concentration was low (20 IU/ml). 
Moreover, the mean difference between the Ct values (3.4 Ct) indicated that the Altona 
RT-PCR may be more sensitive than the Ceeram. But this sensitivity difference could be 
linked to differences in the recommended RNA input volumes: 5µl for the Ceeram assay 
and 25µl for the Altona assay.

The recent evaluation of 2 HEV RNA assays has demonstrated that it is essential 
to use an RT-PCR protocol based on ORF3 in order to accurately quantify all the HEV 
genotype 3 subtypes, as this region is better conserved than most others (16). The 2 
commercial assays tested include primers and a probe targeting this region. However, 
the mean difference between the Ct values for genotype 3e may indicate that the 
Ceeram assay is less sensitive for this subtype. No data are yet available for HEV 
genotypes 1, 2, or 4, but these assays might be suitable for detecting them also, as 
ORF3 is highly conserved across HEV genotypes. These two points should be 
confirmed in further studies.
As transfusion-transmitted HEV3 infections have been reported in industrialized countries (19-22), sensitive HEV RNA assays may well be useful for screening blood products (23). Several studies have reported detecting HEV RNA in pooled plasmas from European blood donors (24-27). Tests using the Altona assay found 1.18% of plasma pools were positive in Germany (24). These two commercial assays now need to be compared for testing plasma pools.

The Ceeram and the Altona assays provide good analytical sensitivity with high reproducibility for detecting genotype 3 HEV RNA. They provide a useful complement to serological methods for detecting HEV infections.
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References


Figure legend

**Figure 1**: Standard curve generated using dilutions of the WHO reference standard. A/ with the Ceeram assay and B/ with the Altona assay. Data are reported as the means of three replicates for each standard dilution.

**Figure 2**: Correlation between Ct values obtained with the Ceeram and Altona assays for the 4 reference strains.
Table 1: Data obtained with the Ceeram and Altona assays for the 4 reference strains

<table>
<thead>
<tr>
<th>Sample (UI/ml)</th>
<th>Ceeram</th>
<th>Altona</th>
<th>Difference Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples detected</td>
<td>Mean Ct</td>
<td>SD</td>
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<tr>
<td>3a 2 500</td>
<td>6/6</td>
<td>34.0</td>
<td>0.5</td>
</tr>
<tr>
<td>500</td>
<td>6/6</td>
<td>36.5</td>
<td>0.6</td>
</tr>
<tr>
<td>100</td>
<td>5/6</td>
<td>38.2</td>
<td>1.1</td>
</tr>
<tr>
<td>20</td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c 2 500</td>
<td>6/6</td>
<td>32.9</td>
<td>0.5</td>
</tr>
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<td>34.6</td>
<td>0.3</td>
</tr>
<tr>
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<td>20</td>
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<td>6/6</td>
<td>33.9</td>
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<td>36.6</td>
<td>0.7</td>
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<tr>
<td>20</td>
<td>5/6</td>
<td>39.2</td>
<td>3.0</td>
</tr>
<tr>
<td>3f 2 500</td>
<td>6/6</td>
<td>33</td>
<td>0.7</td>
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<tr>
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<td>36.3</td>
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<td>20</td>
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<tr>
<td>All samples</td>
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<td>0.9*</td>
<td>2.42**</td>
</tr>
</tbody>
</table>

* | ** | †
Note: SD, standard deviation; CV, coefficient of variation; * Mean standard deviation; ** mean coefficient of variation; †mean Ct difference
Figure 1

A/ Ceeram

\[ y = -3.2257x + 44.891 \]
\[ R^2 = 0.9899 \]

B/ Altona

\[ y = -3.1556x + 42.023 \]
\[ R^2 = 0.9939 \]

Threshold Cycle (Ct) vs. HEV RNA concentration (Log10 IU/ml).
Figure 2

\[ y = 0.8x + 3.4719 \]

\[ R^2 = 0.66 \]