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Performance of Two Commercial Assays for Detecting Hepatitis E Virus RNA in Acute or Chronic Infections

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We assessed the performance of the Ceeram and Altona assays, the first two commercially available hepatitis E virus (HEV) RNA assays, using serial dilutions of 4 HEV-positive reference samples (genotypes 3a, 3c, 3e, and 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 (HEV3); 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 reverse transcription-PCRs (RT-PCRs) were recently evaluated, and their sensitivities were 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 the quantification standard (1 IU/ml corresponds to 1.25 copies/ml) (16). HEV RNA was extracted from blood samples (140 μl) with the RNeasy minikit according to the manufacturer's instructions (Qiagen, Courtaboeuf, France). The HepatitisE@ceeramTools kit by Ceeram (La Chapelle sur Erdre, France) and the RealStar HEV RT-PCR kit, version 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 threshold cycle (C_T) 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 was linear from 20 to 250,000 IU/ml (Fig. 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 C_T values for each dilution. The mean standard deviations were 0.7 C_T (range: 0.4 to 1.6 C_T) for the Ceeram RT-PCR and 0.4 C_T (range: 0.1 to 1.4 C_T) for the Altona RT-PCR.

We assayed samples of strains 3a, 3c, 3e, and 3f to assess analytical sensitivity. The dilution concentrations were 2,500, 500, 100, and 20 IU/ml, and 6 replicates of each were assayed (Table 1). Both assays detected all the 2,500-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 the particular genotype 3 subtype. The Ceeram assay gave higher C_T values than the Altona assay ($P = 0.003$). The mean difference in the C_T values was 3.4 C_T. The differences were 2.9 C_T for subtype 3a, 2.8 C_T for genotype 3c, 5.2 C_T for genotype 3e, and 2.7 C_T for subtype 3f. The mean C_T difference was greater for subtype 3e than for the other subtypes ($P < 0.01$). The Ceeram and the Altona RT-PCR results were correlated ($\rho = 0.88$, $P < 0.001$) (Fig. 2).

A recent evaluation of home brew 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 sensitivities of commercial assays by testing serial dilutions of genotype 3a, 3c, 3e, and 3f reference strains. Sensitivities were 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 C_T values (3.4 C_T) indicated that the Altona RT-PCR may be more sensitive than the Ceeram. But this sensitivity difference could be linked to dif-

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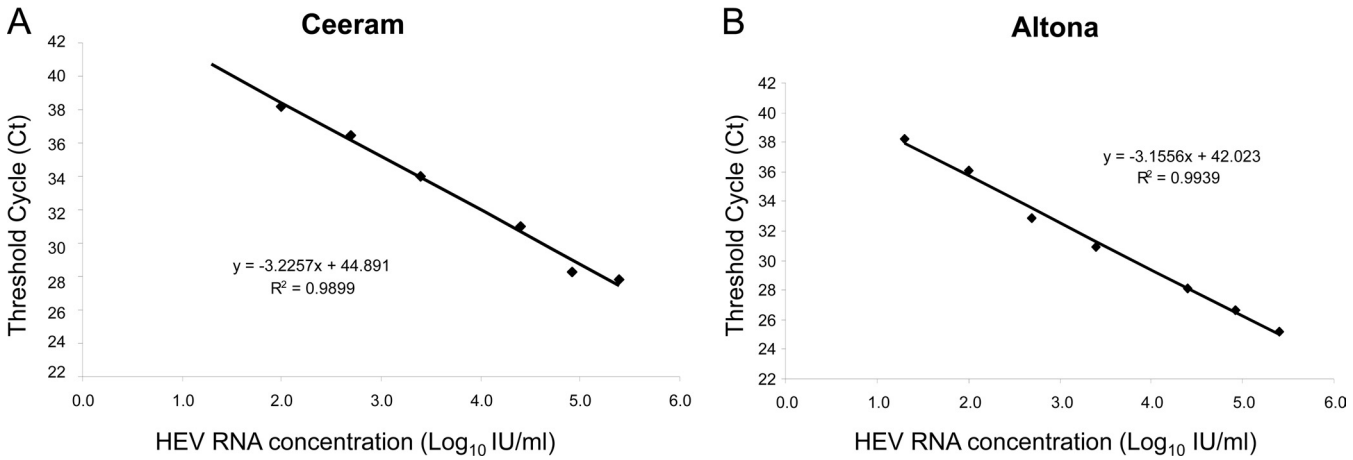

FIG 1 Standard curves generated using dilutions of the WHO reference standard. (A) Ceeram assay. (B) Altona assay. Data are means of three replicates for each standard dilution.

ferences in the recommended RNA input volumes: 5 μl for the Ceeram assay and 25 μl 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 open reading frame 3 (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 C_T 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, and 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). Sev-

TABLE 1 Data obtained with the Ceeram and Altona assays for the 4 reference strains

Subtype	Sample concn (IU/ml)	Result for: Ceeram: No. of samples detected/no. tested	Ceeram: C_T Mean	Ceeram: C_T SD	Ceeram: C_T CV[a] (%)	Altona: No. of samples detected/no. tested	Altona: C_T Mean	Altona: C_T SD	Altona: C_T CV (%)	C_T difference (Ceeram − Altona)
3a	2,500	6/6	34.0	0.5	1.4	6/6	30.9	0.1	0.3	3.1
	500	6/6	36.5	0.6	1.7	6/6	32.9	0.3	0.9	3.6
	100	5/6	38.2	1.1	3.0	6/6	36.0	1.4	3.8	2.1
	20	0/6				4/6	38.3	0.6	1.6	
3c	2,500	6/6	32.9	0.5	1.4	6/6	29.8	0.1	0.3	3.0
	500	6/6	34.6	0.3	0.9	6/6	31.7	0.3	0.9	2.9
	100	6/6	37.5	1.3	3.5	6/6	34.3	1.8	5.2	3.2
	20	1/6	38.4			6/6	36.3	1.0	2.7	2.1
3e	2,500	6/6	32.4	0.3	1.0	6/6	27.4	0.1	0.5	5.0
	500	6/6	33.9	0.3	0.8	6/6	29.4	0.1	0.3	4.6
	100	6/6	36.6	0.7	2.0	6/6	31.3	0.1	0.3	5.3
	20	5/6	39.2	3.0	7.7	6/6	33.3	0.5	1.4	5.9
3f	2,500	6/6	33	0.7	2.1	6/6	30.8	0.2	0.8	2.2
	500	6/6	36.3	0.6	1.7	6/6	32.8	0.7	2	3.5
	100	4/6	37.9	1.6	4.3	6/6	35.9	0.7	2.1	2.0
	20	2/6	39.4			2/6	36.3			3.1
All samples		77/96		0.9[b]	2.42[c]	90/96		0.5[b]	1.5[c]	3.4[d]

[a] CV, coefficient of variation.
[b] Mean standard deviation.
[c] Mean coefficient of variation.
[d] Mean C_T difference.

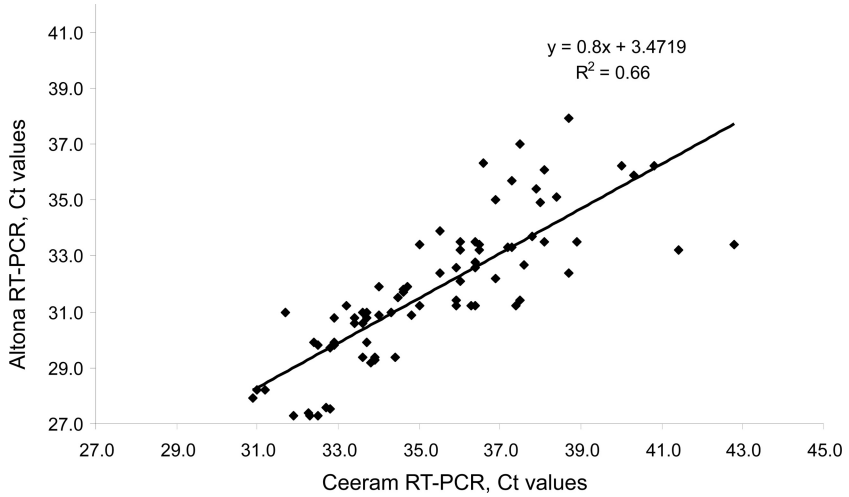

FIG 2 Correlation between C_T values obtained with the Ceeram and Altona assays for the 4 reference strains.

eral studies have reported detecting HEV RNA in pooled plasma samples from European blood donors (24–27). Tests using the Altona assay found that 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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