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NOTES

Good Performance of Immunoglobulin M Assays in Diagnosing Genotype 3 Hepatitis E Virus Infections▽

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We have evaluated three anti-hepatitis E virus (anti-HEV) immunoglobulin M (IgM) assays, the EIAgen HEV IgM assay (Adaltis), the HEV IgM enzyme-linked immunosorbent assay 3.0, and the Assure HEV IgM rapid test (MP Diagnostics), for the routine detection of acute genotype 3 HEV. Their sensitivities were fairly good (90%, 88%, and 82%), and their specificities were excellent (100%, 99.5%, and 100%).

The hepatitis E virus (HEV) is an RNA virus whose genome comprises three open reading frames (ORFs). Four HEV genotypes are identified (12). In countries where HEV is endemic, most of the HEV samples belong to genotype 1 or 2 (9). Autochthonous infections also occur in industrialized countries with genotype 3 HEV (2, 10, 14). Most of the serological assays for diagnosing HEV use recombinant proteins derived from the ORF2 and/or ORF3 proteins. It has been reported that anti-ORF2 antibodies are not specific (13). The sensitivities of the serological assays for immunoglobulin M (IgM) have been found to vary greatly, from 42% to 93% (6, 8, 11, 16). Current commercial serological assays are based on genotype 1 and 2 antigens and thus may be less sensitive for detecting infections with genotype 3 or 4 (4, 15). As there is increasing awareness of HEV in industrialized countries, we have assessed the performance of three serological assays for diagnosing genotype 3 HEV infections: two microplate enzyme immunoassay tests, the EIAgen HEV IgM kit (Adaltis) and the HEV IgM enzyme-linked immunosorbent assay (ELISA) 3.0 (MP Diagnostics), and an immunochromatographic assay, the Assure HEV IgM rapid test (MP Diagnostics).

The sensitivities of the three IgM assays were assessed using 50 HEV RNA-positive samples from French patients with symptoms of acute hepatitis (49 samples with genotype 3 HEV

TABLE 1. Discrepancies between HEV RNA and the different serological test results for the HEV RNA-positive samples[a]

Sample no.	EIAgen HEV IgG assay	EIAgen HEV IgG ratio	EIAgen HEV IgM assay	EIAgen HEV IgM ratio	IgM HEV 3.0 ELISA	IgM HEV 3.0 ELISA ratio	Assure HEV IgM rapid test
1	+	6.5	+	6.9	+	4.66	−
2	+	5.2	+	>10	+	>10	−
3	+	3.03	+	6.3	−	0.64	−
4	+	>10	−	0.8	−	0.59	−
5	−	0.24	+	9.70	+	>10	+
6	−	0.24	+	>10	+	>10	+
7	−	0.23	+	>10	+	2.37	+
8	−	0.93	+	>10	+	>10	+
9	−	0.24	+	>10	+	>10	−
10	−	0.1	−	0.8	−	0.08	−
11	−	0.22	−	0.5	−	0.14	−
12	−	0.1	−	0.2	−	0.14	−
13	−	0.07	−	0.3	−	0.21	−

[a] +, positive result; −, negative result.

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TABLE 2. Serological results for the 50 HEV RNA-positive samples

Anti-HEV IgG test result (no. of samples)	Anti-HEV IgM test result (no. of samples)					
	EIAgen IgM HEV assay (Adaltis)		IgM HEV ELISA 3.0 (MP Diagnostics)		Assure HEV IgM rapid test (MP Diagnostics)	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive (41)	40	1	39	2	37	4
Negative (9)	5	4	5	4	4	5
Total (50)	45	5	44	6	41	9

and 1 sample with genotype 1 HEV). Samples were collected at the onset of the disease. Anti-HEV IgG levels were determined thereafter with the EIAgen HEV IgG kit (Adaltis). Both the anti-HEV IgG assay and the three anti-HEV IgM assays showed positive results for 37 samples. Discrepancies between HEV RNA and the different serological tests were observed for 13 samples (Table 1). Overall, 45 positive samples were obtained by using the EIAgen HEV IgM kit, 44 were obtained by using the HEV IgM ELISA 3.0, and 41 were obtained by using the Assure HEV IgM rapid test (Table 2). The sensitivities of the EIAgen HEV IgM kit, the HEV IgM ELISA 3.0, and the Assure HEV IgM rapid test were 90%, 88%, and 82%, respectively (Table 3). The sensitivities of all three assays were similar ($P > 0.05$).

The specificities of the tests were assessed using 406 HEV RNA-negative samples and anti-HEV IgG-negative samples with the EIAgen HEV IgG kit (Adaltis). All samples were found to be negative by using the EIAgen HEV IgM kit and the Assure HEV IgM rapid test (specificity, 100%), and 404/406 were found to be negative by using the HEV IgM ELISA 3.0 (specificity, 99.5%) (Table 3). The specificities of the three tests were not significantly different ($P > 0.05$). The strength of agreement between the three assays for the 456 samples, evaluated by using the kappa statistic, was excellent. The agreement between the EIAgen HEV IgM assay and the HEV IgM ELISA 3.0 was 99.3%, and that between the EIAgen HEV IgM kit and the Assure HEV IgM rapid test was 99.1% (kappa statistic values, 0.96 and 0.95, respectively; $P < 0.001$). The agreement between the HEV IgM ELISA 3.0 and the Assure HEV IgM rapid test was excellent (98.9%; kappa statistic value, 0.94; $P < 0.001$). The positive and negative delta values (a measure of the number of standard deviations by which the cutoff is separated from the mean of the sample groups [3]) for the two microplate assays were elevated, indicating that they have good abilities to differentiate between infected and noninfected patients (Table 3).

The sensitivity of an anti-HEV IgM test may be influenced by the type of HEV antigen (16). Current commercial serological assays are based on genotype 1 and 2 antigens and thus may be less sensitive for detecting infections with genotype 3 or 4 (4, 15). The HEV IgM ELISA kit (Genelabs Diagnostics), based on genotype 1 and 2 antigens derived from ORF2 and ORF3, had a sensitivity of 53.3% when tested on samples from Taiwan, where HEV is not endemic (8). The EIAgen HEV IgM kit is also based on genotype 1 and 2 synthetic peptides from ORF2 and ORF3, but we found a better sensitivity than that reported for the HEV IgM ELISA kit (Genelabs Diagnostics). The use of a neutralizing agent (goat anti-IgG) in the EIAgen HEV IgM kit to limit the interference from a high concentration of anti-HEV IgG may explain this difference in sensitivity. It has been pointed out that sensitivity is compromised when the corresponding IgG titers are disproportionately higher than those of the IgM antibodies (7). HEV IgM ELISA 3.0 and the Assure HEV IgM rapid test (MP Diagnostics) are based on a conformational ORF2-encoded epitope from a genotype 1 strain, the ORF2.1 antigen, considered highly conserved between HEV strains (1). Their good sensitivities observed in the present work are in keeping with a previous study that demonstrated that the ORF2.1 antigen is suitable for detecting total anti-HEV antibodies in swine infected with genotype 3 (5).

The specificities of all three anti-HEV IgM assays were excellent. However, the selection of the negative samples (HEV RNA negative and IgG negative as determined by the EIAgen IgG HEV kit [Adaltis]) may have influenced the results of the specificity observed for the EIAgen IgM HEV kit (Adaltis) since the IgG and IgM assays are based on the same antigens. Nevertheless, we also used the three anti-HEV IgM assays to retest several samples from healthy French blood donors with negative HEV RNA results and normal alanine aminotransferase results that were considered to be false positive when assayed using the HEV IgM ELISA kit (Genelabs Diagnostics). The two microplate assays decreased the false positive results by 75%, and the immunochromatographic test decreased it by 94%, indicating the high specificity of the three new assays (data not shown).

Serological and molecular tools appear to be complementary for diagnosing HEV infection. Four samples with acute HEV infection were negative for anti-HEV IgG and IgM. Reverse transcription-PCR is very specific and detects HEV RNA early during the acute phase. However, enzyme immu-

TABLE 3. Performance of the three anti-HEV IgM assays[a]

Assay	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)	Positive delta value	Negative delta value
EIAgen HEV IgM (Adaltis)	90 (78.2–96.7)	100 (99.1–100.0)	100 (92.1–100.0)	98.8 (97.0–99.6)	2.63	5.87
HEV IgM 3.0 ELISA (MP Diagnostics)	88 (75.7–95.5)	99.5 (98.0–99.9)	95.7 (85.2–99.5)	98.5 (96.7–99.4)	1.86	4.38
Assure HEV IgM rapid test (MP Diagnostics)	82 (68.6–91.4)	100 (99.1–100.0)	100 (91.4–100.0)	97.8 (95.8–98.9)	ND	ND

[a] PPV, positive predictive value; NPV, negative predictive value; 95% CI, 95% confidence interval; ND, not determined.

noassays are inexpensive and do not require specialized laboratory infrastructures.

The detection of anti-HEV IgM with highly specific assays is useful for diagnosing infections, especially if reverse transcription-PCR is not available. We find that all three assays are suitable tools for the routine detection of acute genotype 3 HEV.

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