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Diagnostic Performance of Five Assays for Anti-Hepatitis E Virus IgG and IgM in a Large Cohort Study

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Determination of anti-hepatitis E virus (anti-HEV) antibodies is still enigmatic. There is no gold standard, and results obtained with different assays often diverge. Herein, five assays were compared for detection of anti-HEV IgM and IgG. Serum samples from 500 Swedish blood donors and 316 patients, of whom 136 had suspected HEV infection, were analyzed. Concordant results for IgM and IgG with all assays were obtained only for 71% and 70% of patients with suspected hepatitis E, respectively. The range of sensitivity for anti-HEV detection was broad (42% to 96%); this was reflected in the detection limit, which varied up to 19-fold for IgM and 17-fold for IgG between assays. HEV RNA was analyzed in all patients and in those blood donors reactive for anti-HEV in any assay, and it was found in 26 individuals. Among all of the assays, both anti-HEV IgG and IgM were detected in 10 of those individuals. Twelve had only IgG and, in 7 of those 12, IgG was only detected with the two most sensitive assays. Three of the HEV-RNA-positive samples were negative for anti-HEV IgM and IgG in all assays. With the two most sensitive assays, anti-HEV IgG was identified in 16% of the blood donor samples and in 66% of patients with suspected HEV infection. Because several HEV-RNA-positive samples had only anti-HEV IgG without anti-HEV IgM or lacked anti-HEV antibodies, analysis for HEV RNA may be warranted as a complement in the laboratory diagnosis of ongoing HEV infection.

Hepatitis E virus (HEV) is transmitted via the fecal-oral route and, globally, is a frequent cause of acute hepatitis. HEV is classified into the *Hepeviridae* family within the genera *Orthohepevirus*. *Orthohepevirus* consists of four species, A through D, infecting mammals and chickens, and *Piscihepevirus* infects trout (1). Four out of seven genotypes of *Orthohepevirus A* are known to infect humans, and genotypes 1 and 2 are endemic in Asia and Africa causing large recurrent outbreaks. Genotype 3 is endemic in Europe, Japan, and the United States. Infection by direct or indirect contact with living animals or with food products contaminated with HEV is probably the most common route of infection with this genotype, but blood transmissions also occur (2).

Hepatitis E virus infection is usually mild or asymptomatic without sequelae, and less than 5% of exposed individuals develop hepatitis (3–5). However, fulminant infection sporadically occurs, and in some patients, chronic infection may ensue, often with rapid fibrosis progression leading to cirrhosis; this most commonly occurs in immunocompromised individuals, such as solid organ recipients and patients receiving chemotherapy (6–11). Additionally, hepatitis E may be associated with neurological manifestations, such as Guillain-Barré syndrome (12), neuralgic amyotrophy (13), and meningitis (14, 15).

Acute, chronic, and past HEV infection can be diagnosed by immunoassays for detection of anti-HEV IgM and IgG in serum as well as by assays for HEV RNA (16). Despite improvements to the assays, their specificities have been difficult to determine, and several studies have evaluated up to seven different assays with anti-HEV serum panels from immunocompetent and immunocompromised patients (17–23). However, many issues remain unresolved regarding the sensitivity and specificity of these assays, and the confirmatory immunoblot is reported unreliable (24). Not surprisingly, discordant results are reported regarding anti-HEV seroprevalence (25, 26).

In the present study, the performances of five commercial assays for the detection of anti-HEV IgM and IgG were compared in a clinical setting using samples from blood donors and patients with liver disease.

MATERIALS AND METHODS

Serum samples. Serum samples from 500 Swedish blood donors sampled at the Department of Transfusion Medicine at Sahlgrenska University Hospital, Gothenburg in 2012 were evaluated. The samples were anonymized with only gender and age known. Another 137 serum samples were derived from patients with suspected hepatitis E that was based on negativity for markers indicative of ongoing hepatitis A, B, or C, who were hospitalized or attending outpatient clinics throughout Sweden. Serum samples from these patients were sent to the Department of Clinical Microbiology/Virology at Sahlgrenska University Hospital, which is the Swedish referral laboratory for hepatitis E. Another 156 patients had liver disease with other etiologies and were attending the Department of Infec-

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TABLE 1 Antigens used for coating and systems used for anti-HEV detection in the different assays

Assay	Antigen for coating	Detection
Mikrogen	Recombinant partial ORF2 and ORF3 genotypes 1 and 3	Labeled anti-human IgG or IgM antibodies
DSI	Recombinant ORF2 and ORF3 peptides cross neutralizing genotypes 1, 2, and 3	Labeled mouse anti-human IgG or IgM antibodies
Euroimmun	Mixture of recombinant partial ORF2 genotype 1 and 3 antigens	Labeled rabbit anti-human IgG or IgM antibodies
Axiom IgG	Recombinant carboxy terminal of ORF2 genotype 1 Burmese strain	Labeled second recombinant ORF 2 antigens
Axiom IgM	Anti-human IgM antibodies	Labeled recombinant ORF 2 antigens
DiaPro	Four synthetic peptides with conservative epitopes of ORF2 and ORF3 in genotypes 1, 2, 3, and 4	Labeled goat anti-human IgG or IgM

tious Diseases or the Department of Internal Medicine at Södra Älvsborgs Hospital in Borås. Serum samples from 23 patients who had undergone liver transplantation at the Transplant Institute at Sahlgrenska University Hospital were also investigated. One to three additional serum samples from 27 patients sampled 1 month to 5 years apart were also analyzed. All serum samples were analyzed at the Department of Clinical Microbiology/Virology. All patients presented with liver disease, and the liver transplant recipients had given their consent to participate in the study, which was approved by the Regional Ethical Review Board in Gothenburg (registration number 737-12).

Enzyme-linked immunosorbent assays. Five commercial assays were used for anti-HEV IgM and IgG detection in the 851 samples. The assays were selected based on routine use in the laboratory and on availability and prior publications (17–20). The selected assays were as follows: (i) recomWell HEV IgG/IgM (Mikrogen Diagnostik, Neuried, Germany), (ii) DS-EIA-ANTI-HEV-G/M (DSI Srl, Milan, Italy), (iii) Anti-Hepatitis E Virus (HEV) ELISA (IgG/IgM) (Euroimmun, Lübeck, Germany), (iv) HEV Ab/HEV IgM EIA (Axiom Diagnostics, Worms, Germany), and (v) HEV IgM/HEV IgG (DiaPro, Milan, Italy). DS-EIA corresponds to an assay produced by RPC Diagnostic Systems (Nizhny Novgorod, Russia), and the Axiom assay corresponds to an assay produced by Wantai (Beijing, China). The formats of the assays are given in Table 1. All serum samples were kept frozen at −20°C until tested for anti-HEV IgM and IgG in parallel. The cutoff of each assay was calculated according to the manufacturer's instructions, and the optical density (OD) value for each sample was divided by the cutoff value of the assay (sample OD/cutoff OD of the assay). All samples with OD values above or at the cutoff were reanalyzed in duplicate for that particular assay, and the reactivity of the sample was determined by the mean OD value of the reanalyzed duplicates. Due to the highly different numbers of anti-HEV IgG-reactive samples in different assays, the cutoff values were analyzed with the receiver operating characteristic (ROC) curve using GraphPad Prism version 6.04 (GraphPad Software Inc., CA, USA).

To determine the detection limit, serial two-step dilutions (from 1/2 to 1/64) of the WHO reference reagent for hepatitis E virus (National Institute for Biological Standards and Control [NIBSC] code 95/584 [27]) containing 100 IU/ml anti-HEV IgG and IgM were performed in anti-HEV and HEV-RNA-negative serum. Two serum samples that were positive for anti-HEV IgM and IgG in all assays were also diluted in negative serum. Each dilution was divided into six aliquots, and these were frozen at −20°C and thawed only once before being analyzed in duplicate. For determination of endpoint titers, linear regression was performed on the log(mean OD/cutoff OD) of each dilution versus the log(dilution of the sample) using the GraphPad Prism program.

HEV RNA detection by real-time PCR. All blood donor samples that were reactive for anti-HEV IgG and/or IgM in any assay and all patient samples were analyzed for HEV RNA. The RNA was extracted from a 250-μl serum sample mixed with 2 ml lysis buffer (NucliSENS easyMag; bioMérieux SA, France). The mixture was incubated for 10 min at room temperature before the addition of 50 μl of NucliSENS easyMag magnetic silica and was incubated for an additional 10 min. RNA was eluted in 110 μl distilled water by using the NucliSENS easyMag instrument according to the manufacturer's instructions (bioMérieux SA, France).

Real-time PCR was performed on 20 μl extracted RNA in 30 μl master mix consisting of 25 μl 2× reaction mix with ROX, 1 μl SuperScript III RT/Platinum *Taq* mix, 1 μl RNaseOUT (Invitrogen), and 200 nM each of the forward primer (JVHEVF), reverse primer (JVHEVR), and probe (HEVP). Cycling conditions were performed as previously described (28).

Statistical analysis. All statistical analyses were performed using the GraphPad Prism version 6.04 (GraphPad software Inc., CA, USA). Specificity and sensitivity of the assays were calculated according to Baratloo et al. (29).

RESULTS

There were considerable differences in reactivity when using the recommended cutoffs at the initial testing of the material. The highest reactivity was obtained using DiaPro with 49 serum samples that were reactive for anti-HEV IgM and 255 samples that were reactive for anti-HEV IgG. The lowest reactivity was noted with Euroimmun, with 16 and 79 serum samples reactive for anti-HEV IgM and IgG, respectively (see Fig. S1 in the supplemental material). The values of the log(sample OD/cutoff OD of the assay) obtained for each sample and for each patient category from two assays were plotted on a scatter plot, with the values obtained with one assay on the *x* axis and the values obtained with another assay on the *y* axis (see Fig. S1). Because the plot of Mikrogen and Euroimmun formed a straight line below the origin, these two assays appeared to have similar reactivity for each sample. However, the cutoff seemed to be set too low for Euroimmun (see Fig. S1). Most samples with low levels of reactivity slightly above the cutoff were obtained with DiaPro. Therefore, all assays were subject to ROC curve analyses, and based on these results, optimal cutoff values of 0.8 for Euroimmun and 1.3 for DiaPro for anti-HEV IgG were found and used in further analyses (see Table S1 in the supplemental material). Otherwise, the recommended cutoffs were used.

After adjustment of the cutoff values, reactivity in two or more assays for the same marker (i.e., anti-HEV IgG or IgM) was considered a specific outcome. Hepatitis E virus infection was also considered confirmed for patients with serum samples reactive for both IgG and IgM with different assays or with positive results in HEV PCR.

Anti-HEV IgM. Analysis of the dilution series of the WHO reference reagent and two patient serum samples revealed a detection limit from 5.1 to 23.8 IU/ml, with Axiom and DiaPro being the most sensitive (Table 2; see also Fig. S2 in the supplemental material). There was also an up to 19-fold difference in the detection limit for the diluted patient serum samples, with Axiom being the most sensitive followed by DiaPro and DSI (Table 2).

For 749 (92%) of the 816 samples, there were concordant results for anti-HEV IgM with all five assays (see Table S2 in the supplemental material). IgM reactivity was confirmed for 68 of

TABLE 2 Detection limits and endpoint titers obtained from analyzing the dilution series of the WHO reference reagent 95/584 and two patient serum samples in five anti-HEV assays

Reference reagent or patient sample	Detection limit for assay:				
	Mikrogen	DSI	Euroimmun	Axiom	DiaPro
Anti-HEV IgM					
WHO 95/584 100 IU/ml (IU/ml)	16	8.5	24	5.1	5.4
Sample 17176/13	1/53	1/220	1/35	1/270	1/140
Sample 4290/14	1/43	1/110	1/22	1/420	1/360
Anti-HEV IgG					
WHO 95/584 100 IU/ml (IU/ml)	0.9	0.4	2.2	0.2	0.2
Sample 17176/13	1/86	1/190	1/27	1/430	1/480
Sample 4290/14	1/230	1/480	1/130	1/630	1/880

the 80 IgM-reactive samples (85%) by anti-HEV IgM reactivity with more than one assay and/or with anti-HEV IgG or HEV RNA (Table 3). Forty-six of these 68 confirmed anti-HEV IgM samples were from patients with suspected hepatitis E (Table 3). The sensitivity for anti-HEV IgM detection was low and varied from 24% for the Euroimmun assay to 72% for the DiaPro assay.

Anti-HEV IgG. The detection limit for anti-HEV IgG was determined as for anti-HEV IgM and revealed up to 11-fold differences between the assays (from 0.2 to 2.2 IU/ml) and up to 17-fold differences in the endpoint titers of the diluted serum samples, with the Axiom and DiaPro assays being the most sensitive (Table 2).

More samples were reactive for anti-HEV IgG than for IgM. Concordant results were obtained for 644 (79%) of the 816 samples (see Table S3 in the supplemental material). The highest concordance in anti-HEV IgG reactivity was found with the Axiom and DiaPro assays and was 99% for serum samples from patients with suspected hepatitis E and 96% for all serum samples.

There were 216 samples with confirmed anti-HEV IgG reactivity. All 22 HEV-RNA-positive samples with anti-HEV IgG were reactive with Axiom and DiaPro (Table 3). The highest number of anti-HEV IgG singly reactive samples was obtained with DiaPro, with 22 out 235 positive serum samples in just one assay. The lowest reactivity was obtained with Euroimmun, which had 94 reactive samples; 91 of those were confirmed, and 15 of those were confirmed by the presence of HEV RNA. The sensitivity for anti-HEV IgG detection was higher than that for IgM detection and varied from 42% with Euroimmun to 98% with DiaPro (Table 3).

HEV RNA. Five blood donor samples and 21 patient samples had detectable HEV RNA (Table 4). Ten of these 26 samples were reactive for anti-HEV IgM and IgG in all five assays. For the other 16 HEV-RNA-containing samples, 12 were reactive for anti-HEV IgG. Four of these were reactive for anti-HEV IgG with all five assays, one was reactive with all of the assays except Microgen, and seven were only reactive with Axiom and DiaPro.

Blood donor serum samples. The mean age of the 500 blood

TABLE 3 Reactivity for anti-HEV IgG and IgM in respective assays for 66 serum samples confirmed reactive for anti-HEV IgM, 12 of which were positive for HEV RNA, and for 216 serum samples confirmed reactive for anti-HEV IgG, 22 of which were positive for HEV RNA

Assay and reactivity	No. HEV IgM (HEV RNA positive)	No. HEV IgG (HEV RNA positive)	Sensitivity IgM/IgG (%)	Specificity IgM/IgG (%)
Mikrogen				
Reactive also in other assay(s)	22 (9)	134 (14)	38 IgM	99 IgM
Confirmed singly reactive	4[a] (1)	0	62 IgG	99 IgG
Not confirmed singly reactive	9	4		
DSI				
Reactive also in other assay(s)	33 (9)	156 (14)	63 IgM	99 IgM
Confirmed singly reactive	10[b] (1)	1[c] (0)	72 IgG	99 IgG
Not confirmed singly reactive	3	3		
Euroimmun				
Reactive also in other assay(s)	16 (10)	91 (15)	24 IgM	100 IgM
Confirmed singly reactive	0	0	42 IgG	99 IgG
Not confirmed singly reactive	0	4		
Axiom				
Reactive also in other assay(s)	19 (10)	203 (22)	29 IgM	99 IgM
Confirmed singly reactive	1[d] (0)	3[e] (0)	95 IgG	98 IgG
Not confirmed singly reactive	1	9		
DiaPro				
Reactive also in other assay(s)	31 (10)	209 (22)	72 IgM	100 IgM
Confirmed singly reactive	18[f] (1)	2[g] (0)	98 IgG	96 IgG
Not confirmed singly reactive	0	22		

[a] Four serum samples were reactive for anti-HEV IgG, two with all of the assays and one each with DSI and DiaPro.
[b] Ten serum samples were reactive for anti-HEV IgG, five with all of the assays, two with Axiom and DiaPro, two with Axiom only, and one with DiaPro only.
[c] One serum sample was positive for anti-HEV IgM with Microgen.
[d] One serum sample was positive for anti-HEV IgG with all five assays.
[e] Three serum samples were positive for anti-HEV IgM, one with DSI, Axiom, and DiaPro, and two with DSI only.
[f] All serum samples were positive for anti-HEV IgG, 17 with all five assays and one with Axiom and DiaPro.
[g] Two serum samples were positive for anti-HEV IgM, one with Microgen and one with DSI.

TABLE 4 Summarized results for anti-HEV and HEV RNA for 500 blood donors, 137 patients with suspected hepatitis E, 156 patients with other forms of liver disease, and 23 liver transplant recipients[a]

Group	No. (%)	No. (%) HEV IgM reactive	No. (%) HEV IgG reactive	No. (%) HEV RNA reactive
Blood donors	500			5 (1)
Positive for two or more HEV markers or HEV RNA only	81 (16)	13 (2.6)	81 (16)	5
Reactive in one assay only	29 (6)	4	25	0
Negative for HEV markers	390 (78)			
Suspected hepatitis E	137			16 (12)
Positive for two or more HEV markers or HEV RNA only	93 (68)	45 (33)	91 (66)	16
Reactive in one assay only	7 (5.1)	5	2	0
Negative for HEV markers	37 (27)			
Other liver disease	156			3 (2)
Positive for two or more HEV markers or HEV RNA only	39 (25)	8 (5.1)	38 (24)	3
Reactive in one assay only	14 (9)	2	12	0
Negative for HEV markers	103 (66)			
Liver transplant recipients	23			2 (9)
Positive for two or more HEV markers or HEV RNA only	7 (30)	0	6 (26)	2
Reactive in one assay only	4 (17)	1	3	0
Negative for HEV markers	12 (53)			
Total	816			26 (3)
Positive for two or more HEV markers or HEV RNA only	220 (27)	66 (8)	216 (26)	26 (3)
Reactive in one assay only	54 (7)	12 (1.5)	42 (5)	0
Negative for HEV markers	542 (66)			

[a] Samples reactive for one of the markers anti-IgG or IgM in more than one assay or only reactive for HEV RNA are regarded as having an acute or as having had a past HEV infection.

donors was 42 years, 44 years for males (range, 18 to 75) and 41 years for females (range, 19 to 74). The seroprevalence of anti-HEV IgG varied from 5% to 19% depending on the assay used and was 16% when confirmed by reactivity in two assays (17% for males and 13% for females). There was a significantly higher seroprevalence in donors older than 50 years for the two sexes, 57/170 (34%) versus 16/330 (5%) ($P < 0.001$; Fisher's exact test).

Patient serum samples. A total of 216 (26%) of the samples had hepatitis E markers (i.e., anti-HEV IgM, IgG, and/or HEV RNA). Anti-HEV IgM was identified in 66 (31%) of those samples (Tables 3 and 4). Among the 316 patients, the seroprevalence of anti-HEV IgG was more than twice as high in patients older than 50 years (99/187, 53%) compared to those younger than 50 years (32/129, 25%) ($P < 0.0001$; Fisher's exact test). There was no difference in prevalence between the genders, with seroprevalences of 66/171 (38%) among males and of 65/145 (45%) among females ($P = 0.30$; Fisher's exact test). Anti-HEV IgM and HEV RNA were equally prevalent in patients younger and older than 50 years (9% and 8% versus 8% and 6%) with no gender difference (see Fig. S3 in the supplemental material).

The 137 patients with suspected hepatitis E had the highest anti-HEV prevalence, and 93 (68%) had more than one marker for hepatitis E indicating ongoing infection (Table 5). Another 32% had confirmed anti-HEV IgG indicating recent HEV infection. The patients with suspected HEV infection had the highest rate of confirmed HEV IgM reactivity (46/66; 68%). Forty-two of 45 patients (93%) were reactive with DiaPro followed by 27/45 (60%) reactive with DSI. Most HEV-RNA-positive serum samples in this group (14/16) had detectable HEV markers with DiaPro and Axiom assays followed by the DSI assay (13 serum samples) (Table 5). Additionally, patients with liver disease (39/156, 25%) and liver transplant recipients (7/23; 30%) had a higher seroprevalence against hepatitis E than blood donors ($P = 0.02$; Fisher's exact test) (Table 4). The sensitivity for detection of past or present HEV infection in all patient groups varies from 43% to 97% (Table 5), and the specificity varies from 96.6% to 99.5% (Table 6) between the assays.

Follow-up serum samples. To investigate possible differences due to time between the assays in the detection of anti-HEV IgG or IgM, serial samples were investigated for 27 patients, 15 of whom showed the same reactivity with all assays in serum samples drawn 2 weeks to 2 years apart (see Table S4 in the supplemental material). Anti-HEV IgM was shown to persist in patient 1 for at least 7 months with all assays, in patient 23 for at least 5 months with the Axiom assay, and in patient 13 for 2 months with the DiaPro assay. Patient 115 was consistently reactive for anti-HEV IgM in two serum samples drawn 2 weeks apart with the Mikrogen assay. Anti-HEV IgG was shown to persist with all assays for at least 5 years in patient 51 and for at least 1 year in three other patients.

DISCUSSION

In our comparison of anti-HEV IgM assays, concordant results were obtained with 71% to 81% of the serum samples from patients with suspected hepatitis E. This indicates that most of these assays can diagnose acute HEV infection because generally high titers of anti-HEV IgM are present before the onset of symptoms. The level of anti-HEV IgM decreases with time and becomes undetectable 4 to 6 months after acute infection (16, 30). Anti-HEV IgM may therefore become undetectable earlier after an acute infection when using the less sensitive assays. Sensitivity for anti-HEV IgM varied up

TABLE 5 Sensitivity for anti-HEV IgG and/or IgM detection in 221 patients with confirmed ongoing or passed hepatitis E, i.e., positive for two or more HEV markers or HEV RNA

Patient group and assay (no. of patients with HEV markers)	HEV IgM positive; % (HEV RNA positive)	HEV IgG positive; % (HEV RNA positive)	IgM and/or IgG positive; % (HEV RNA positive)
Blood donors (82)	13 (1)	82 (5)	82 (5)
DiaPro	7; 54 (1)	80; 98 (5)	81; 99 (5)
Axiom	3; 23 (0)	76; 93 (5)	76; 93 (5)
DSI	11; 85 (0)	51; 62 (1)	56; 68 (1)
Mikrogen	1; 8 (0)	33; 40 (0)	33; 40 (0)
Euroimmun	1; 8 (0)	23; 28 (1)	23; 28 (1)
Confirmed hepatitis E (93[a])	45 (12)	91 (15)	92 (15)
DiaPro	42; 93 (10)	90; 99 (14)	90; 97 (14)
Axiom	15; 33 (10)	90; 99 (14)	90; 97 (14)
DSI	27; 60 (11)	77; 85 (12)	77; 83 (13)
Mikrogen	19; 42 (11)	74; 81 (12)	74; 80 (12)
Euroimmun	15; 33 (10)	54; 59 (12)	55; 59 (12)
Other liver disease (39[a])	8 (0)	38 (2)	38 (2)
DiaPro	0	35; 92 (2)	35; 90 (2)
Axiom	1; 12 (0)	33; 87 (2)	33; 84 (2)
DSI	6; 75 (0)	25; 66 (1)	26; 67 (1)
Mikrogen	4; 50 (0)	23; 61 (1)	25; 64 (1)
Euroimmun	0	11; 29 (1)	11; 28 (1)
Liver transplanted (7[a])	0	6 (1)	6 (1)
DiaPro	0	6 (1)	6; 86 (1)
Axiom	0	6 (1)	6; 86 (1)
DSI	0	4 (1)	4; 57 (1)
Mikrogen	0	4 (1)	4; 57 (1)
Euroimmun	0	4 (1)	4; 57 (1)
Total (221)	66 (13)	217 (22)	218 (23)
DiaPro	49; 75 (11)	210; 97 (22)	211; 97 (22)
Axiom	19; 29 (10)	205; 94 (22)	205; 94 (22)
DSI	44; 67 (11)	153; 71 (15)	163; 75 (16)
Mikrogen	24; 36 (11)	134; 62 (14)	136; 62 (14)
Euroimmun	16; 24 (10)	92; 42 (15)	93; 43 (15)

[a] One patient has HEV RNA only as a marker for HEV infection.

to 19-fold between the assays, which may be reflected in the observed persistence of anti-HEV IgM, which varied from a few weeks to 3 months. However, follow-up samples were available for only a few patients. All samples that were single reactive for anti-HEV IgM with the DiaPro assay were also reactive for other hepatitis E markers, suggesting that the more sensitive assays may diagnose a hepatitis E virus infection for a longer period after onset. This is important for the clinician because most patients are sampled rather late after the onset due to lack of awareness among physicians in Nordic countries of endemic hepatitis E. Therefore, a higher sensitivity for anti-HEV IgM may not always result in a higher number of early diagnosed infections. Most of the singly reactive and unconfirmed anti-HEV IgM results were obtained with the Mikrogen and DSI assays, which may indicate nonspecific reactions. It may also indicate that these assays detect preformed anti-HEV IgM due to immature B lymphocytes unrelated to previous exposure to HEV, as has been shown for IgM against hepatitis B virus core antigen (31).

Acute hepatitis E may be diagnosed not only by IgM but also by HEV RNA, which is detectable 1 to 2 weeks before the onset of symptoms and persists for 1 week in serum and for another 2 weeks in feces (16). Due to this short duration of the viremic period, undetectable HEV RNA may not exclude acute hepatitis E. Although HEV RNA was detected in 10 samples that were highly reactive for anti-HEV IgM with all five assays, HEV RNA was also present in 13 samples lacking anti-HEV IgM, which indicates that there may be either a weak response that is not detected by the most sensitive assays or perhaps that there is a lack of IgM response in some patients. Our results indicate that patients with elevated transaminases or suspected ongoing HEV infection should be tested for anti-HEV and HEV RNA. Previously, nonreactivity for anti-HEV has been reported in Swedish blood donors with 1 per 7,980 donations having detectable HEV RNA, most without other HEV markers (32).

Anti-HEV IgG appears shortly after IgM, increases from the acute through the convalescent phase, and may be detected up to 4.5 years after acute infection (18, 33). When comparing the endpoints of a dilution series, the sensitivity of anti-HEV IgG detection with the compared assays differed by up to 17 times. This was reflected when testing samples for anti-HEV IgG with 70% to 99% concordant results, which is important not only for the determination of previous exposure to HEV or seroprevalence of anti-HEV in different populations but also for the detection of replicating HEV infection as shown by the presence of HEV RNA. It is notable that 7 of the 12 samples with HEV RNA and anti-HEV IgG were reactive only with the Axiom and DiaPro assays. The high prevalence of HEV RNA in samples with

TABLE 6 Specificity for anti-HEV IgG and IgM detection based on 595 sera from patients without markers for HEV infection or reactive in only one assay and regarded as nonspecific.

Patient group (no.) and assay	No. nonreactive for HEV IgM (%)	No. nonreactive for HEV IgG (%)	No. nonreactive for both markers (%)
Blood donors (418)			
DiaPro	418 (100)	403 (96.4)	403 (96.4)
Axiom	418 (100)	414 (99.0)	414 (99.0)
DSI	417 (99.8)	417 (99.8)	416 (99.5)
Mikrogen	415 (99.3)	418 (100)	412 (98.6)
Euroimmun	418 (100)	417 (99.8)	417 (99.8)
Suspected hepatitis E (44)			
DiaPro	44 (100)	43 (97.7)	43 (97.7)
Axiom	43 (97.7)	44 (100)	43 (97.7)
DSI	43 (97.7)	44 (100)	43 (97.7)
Mikrogen	41 (93.2)	44 (100)	41 (93.2)
Euroimmun	44 (100)	43 (97.7)	43 (97.7)
Other liver disease (117)			
DiaPro	117 (100)	113 (96.6)	113 (96.6)
Axiom	117 (100)	113 (96.6)	113 (96.6)
DSI	117 (100)	115 (98.3)	115 (98.3)
Mikrogen	115 (98.3)	116 (99.1)	114 (97.4)
Euroimmun	117 (100)	116 (99.1)	116 (99.1)
Transplant recipients (16)			
DiaPro	16 (100)	15 (93.8)	15 (93.8)
Axiom	16 (100)	15 (93.8)	15 (93.8)
DSI	16 (100)	16 (100)	16 (100)
Mikrogen	15 (93.8)	16 (100)	15 (93.8)
Euroimmun	16 (100)	15 (93.8)	15 (93.8)
Total (595)			
DiaPro	595 (100)	574 (96.5)	575 (96.6)
Axiom	594 (99.8)	586 (98.5)	586 (98.5)
DSI	593 (99.7)	592 (99.5)	591 (99.3)
Mikrogen	586 (98.5)	594 (99.8)	583 (98.0)
Euroimmun	595 (100)	591 (99.3)	592 (99.5)

only detectable anti-HEV IgG may indicate a low sensitivity for the detection of anti-HEV IgM during acute infection.

The assays may include viral antigens common for only one or two genotypes and, due to considerable cross-reactivity between the genotypes, are able to detect antibodies directed against other genotypes. The compared assays had similar recombinant antigens, although Axiom differed by having labeled recombinant HEV antigens instead of labeled anti-human antibodies as used in the other assays. Additionally, the DiaPro assay was based on peptides corresponding to open reading frame 2 (ORF2) and three of the genotypes 1 to 4. This may, to some extent, explain the higher sensitivity with these assays.

The divergent positive results with the less sensitive assays indicate nonspecific reactivity because 5% of the serum samples were reactive for anti-HEV IgG in just one assay, and none of these singly reactive samples had detectable HEV RNA. Singly reactive serum samples with either of the two most sensitive assays may have had nonspecific results. However, this was not the case because the majority of singly reactive results with these assays could be confirmed by another HEV marker. There were concordant results with DiaPro and Axiom for IgG in 99% of the samples from patients with suspected hepatitis E, so any of these assays may be used to confirm the result obtained by the other. Despite the high sensitivity for anti-HEV detection, there were serum samples from immunocompetent patients with HEV RNA lacking serological markers for HEV. The significance of this finding needs further investigation.

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