

# Test Performance Characteristics of Anti-HEV IgG Assays Strongly Influence Hepatitis E Seroprevalence Estimates

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Hepatitis E virus (HEV) seroprevalences of 0.3%–53% were reported from industrialized countries. Because these estimates may be influenced by detection assays, this study compares 3 frequently used tests for HEV detection: the MP Diagnostics HEV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA), the Axiom Diagnostics HEV IgG enzyme immunoassay (EIA), and the Mikrogen *recomLine* HEV IgG assay. Sera from 200 healthy healthcare workers and 30 individuals with acute HEV infection were analyzed. Among the healthy individuals, HEV IgG was found in 4.5% by the MP Diagnostics assay, in 29.5% by the Axiom Diagnostics assay, and in 18% by the Mikrogen assay. Among individuals with acute HEV infection, positive results were obtained for 83.3%, 100%, and 96.7%, respectively. Thus, the 3 assays show clear differences in diagnostic sensitivity.

**Keywords.** Hepatitis E; HEV; anti-HEV IgG; seroprevalence; serology; diagnostic sensitivity; commercial assays; acute infection; southeastern Germany.

Hepatitis E virus (HEV) is a small, nonenveloped RNA virus. Analysis of viral genomes led to the identification of 4 mammalian HEV genotypes with distinct geographic distributions

[1]. The virus was first postulated in 1980 as causative agent of enterically transmitted non-A, non-B hepatitis in India and was identified 3 years later. In the following years, genotype 1 HEV was, in many developing countries, identified as a major cause of infectious hepatitis transmitted by the fecal-oral route [1].

In recent years, studies have documented that HEV infection also occurs among individuals in industrialized countries with no history of travel to HEV-endemic areas [2–4]. Because viral isolates from these cases were almost identical to strains detected in swine and wild boars, zoonotic sources of infection are suspected [5–7]. In Germany, the majority of HEV strains isolated from patients with acute hepatitis E were genotype 3 [4, 7].

Prevalence estimates for HEV antibodies in different European populations range from 0.3% to 52.5%. Low seroprevalences (<4%) were reported from Greece, the Netherlands, Italy, and northern France, whereas high seroprevalences (>16%) were reported from the United Kingdom, Denmark, Moldova, and southwest France [8]. This high variability is likely attributable to the cultural background and dietary habits of the study population. However, the diagnostic sensitivity of the HEV antibody assays used in different studies might also influence the results and, possibly, be the most important cause for the striking differences.

This study investigates whether different detection assays yield different estimates of HEV antibody prevalence. We compared 3 commercially available HEV immunoglobulin G (IgG) assays by analyzing sera from healthy healthcare workers in southeastern Germany. We further determined how the assays performed in the detection of acute hepatitis E by analyzing specimens from 30 individuals who were polymerase chain reaction (PCR) positive for HEV.

## METHODS

### Sample Collection

Serum samples were collected during routine daily operations of our diagnostic laboratory in 2010. Most specimens were pseudonymized sera collected from healthy healthcare workers at the University Hospital of Regensburg during their routine appointment with the company medical officer. A total of 200 samples were analyzed: 100 were from males, and 100 were from females. All subjects resided in southeastern Germany; 29 were aged 15–24 years, 39 were aged 25–34 years, 46 were aged 35–44 years, 48 were aged 45–54 years, and 38 were aged 55–65 years. Thirty samples from patients with acute hepatitis

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E were also tested; 23 patients were male, 7 were female, and the median age was 43 years (range, 12–75 years). Acute hepatitis E was diagnosed on the basis of clinical symptoms and/or serological findings (ie, detection of anti-HEV immunoglobulin M [IgM]). Laboratories used the following screening assays to diagnose acute hepatitis E: the *recomLine* HEV immunoblot (Mikrogen; Neuried, Germany), in 54% of cases; the *recomWell* HEV enzyme-linked immunosorbent assay (ELISA; Mikrogen), in 23%; and an HEV ELISA (MP Diagnostics; Illkirch, France), in 23%. All cases were confirmed by quantitative reverse transcription PCR in our laboratory, as described previously [4, 7]. HEV genotype 3 was detected in 23 patients, and HEV genotype 1 was detected in 3; the HEV genotype in 4 was unknown. Additional details about patients with acute hepatitis E can be found in [Supplementary Table 3](#).

### Characteristics of Anti-HEV IgG Assays

Serum samples were tested for HEV IgG antibodies, using the following 3 commercially available immunoassays: the MP Diagnostics HEV IgG ELISA (developed by Genelabs Technologies; Singapore), the Axiom Diagnostics (Worms, Germany) HEV IgG EIA (developed by Wantai; Beijing, China), and the *recomLine* HEV IgG immunoblot, denoted hereafter as assay A, assay B, and assay C, respectively. All assays were performed according to the manufacturer's instructions. In brief, for each ELISA measurement, a signal-to-cutoff ratio (SCR) was calculated by dividing the optical density (OD) by the respective cutoff OD. A negative result was recorded for SCRs of <0.9, a borderline result was recorded for SCRs of 0.9–1.1, and a positive result was recorded for SCRs of >1.1. These criteria were used for assays A and B to achieve better comparability. Assay C is based on 7 recombinant antigens of HEV genotypes

1 and 3. Stained test strips were scanned with the *recomScan* analysis software (Mikrogen). Each antigen band with an intensity greater than or equal to the cutoff was assigned a predefined point value. The final results were classified into 3 categories: negative, defined as  $\leq 2$  points; borderline, defined as 3 points; and positive, defined as  $\geq 4$  points.

## RESULTS

### Samples From Healthy Healthcare Workers

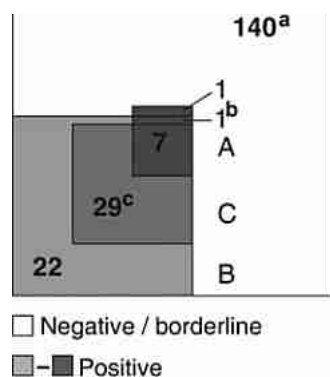
All 200 samples from the healthy healthcare workers underwent analysis by each anti-HEV IgG assay. Nine samples (4.5%) yielded positive results by assay A, compared with 59 (29.5%) by assay B and 36 (18%) by assay C. Thus, the overall seroprevalence suggested by assay B was >6.5 times higher than that suggested by assay A. Borderline results were yielded in 3 cases (1.5%) by assays A and B and in 5 cases (2.5%) by assay C. With all assays, there was a trend of increasing seroprevalence with age ([Supplementary Figure 1](#)). Positive results were yielded for 7 samples (3.5%) by all assays, for 29 (14.5%) by assays B and C only, and for 22 (11.0%) by assay B only ([Supplementary Table 2](#)). Analysis of the distribution of results demonstrated that the 7 samples positive in all assays were from a subset of 36 samples that were positive by assay C ([Figure 1](#)). These 36 samples were from a subset of 59 samples that were positive by assay B ([Figure 1](#)). Negative results in all assays were found for 132 samples (66%).

The correlation of SCRs for each assay combination is shown in [Figure 2](#). There was little correlation between SCRs in assays A and B ([Figure 2A](#)). Similarly, there was little correlation between SCRs in assay A and points in assays C ([Figure 2B](#)). A roughly better correlation was found between points in assay C and SCRs in assay B ([Figure 2C](#)).

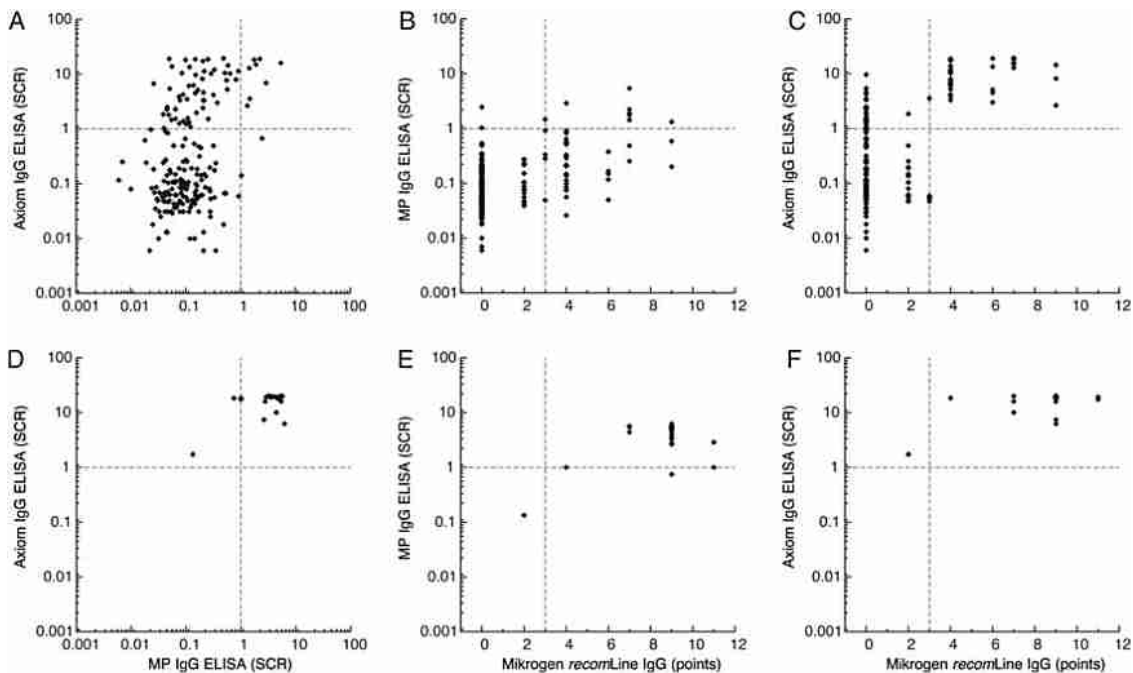
### Samples From Individuals With Acute Hepatitis E

Thirty sera from patients with acute hepatitis E were analyzed by each anti-HEV IgG assay. A total of 25 samples (83.3%) were positive by assay A, compared with 30 (100%) by assay B and 29 (96.7%) by assay C. Borderline results were yielded in 2 cases (6.7%) by assay A. Positive results were yielded for 25 samples (83.3%) by all assays, for 2 (6.7%) by assays B and C only, and for 1 (3.3%) by assay B only ([Supplementary Table 2](#)). There was good correlation between SCRs in assays A and B ([Figure 2D](#)). However, correlation was lower between points and SCRs in the remaining assay comparisons ([Figure 2E](#) and [2F](#)).

Sera from this group were retested with the IgM versions of assay B and C. Because some samples were depleted, results for only 28 specimens (for assay B) and 29 specimens (for assay C) were available. All 28 specimens (100%) tested by the IgM version of assay B were positive, whereas 25 of 29 (86.2%) tested by the IgM version of assay C were positive; 4



**Figure 1.** Schematic distribution of positive results generated with the MP Diagnostics hepatitis E virus (HEV) immunoglobulin G (IgG) enzyme-linked immunosorbent assay (A), the Axiom Diagnostics HEV IgG enzyme immunoassay (B), and the Mikrogen *recomLine* HEV IgG assay (C). Borderline results are ignored (ie, rated negative). <sup>a</sup>Contains 8 samples with borderline results. <sup>b</sup>One sample had a borderline result of assay C. <sup>c</sup>One sample had a borderline result of assay A.



**Figure 2.** Relationship of signal-to-cutoff ratios (SCRs) and total points among the MP Diagnostics hepatitis E virus (HEV) immunoglobulin G (IgG) enzyme-linked immunosorbent assay, the Axiom Diagnostics HEV IgG enzyme immunoassay, and the Mikrogen *recomLine* HEV IgG assay. Broken lines indicate the borderline regions (0.9–1.1 SCRs for ELISAs and 3 points for the immunoblot) between negative results (<0.9 SCR and ≤2 points, respectively) and positive results (>1.1 SCR and ≥4 points, respectively).

of 29 specimens (13.8%) tested by this version of assay C had borderline results. The 5 sera (16.7%) that were negative or of borderline positivity for HEV IgG by at least 1 of the 3 assays were positive by the IgM version of assays B and C.

## DISCUSSION

This study shows significant differences in diagnostic sensitivities of 3 commercially available anti-HEV IgG assays. When these assays are used in seroprevalence studies, they may lead to dramatic differences in the estimated percentage of anti-HEV-positive individuals in a population.

In our study, we analyzed sera from 200 healthy healthcare workers from the University Hospital of Regensburg and 30 sera from individuals with acute hepatitis E. In the population of healthcare workers, we found 4.5%, 18.0%, and 29.5% to be positive for anti-HEV IgG by assays A, C, and B, respectively.

In a first attempt at an explanation of these discrepant results, one might assume that low specificities of assays C and B yielded the higher positive rates. However, an analysis of the results demonstrated that 7 of the 9 samples positive by assay A were a subset of the 36 sera positive by assay C, the latter in turn being a subset of the 59 samples positive by assay B. Random results due to a lack of specificity would not

show such a pattern (at least not for assays A and C). For this phenomenon, a difference in diagnostic sensitivity is the most possible explanation. The findings in sera from patients with acute hepatitis E point in the same direction. Here, the 3 assays showed more or less comparable results, with 83.3%, 96.7%, and 100% of specimens testing positive by assays A, C, and B, respectively. These findings are consistent with the ability of less sensitive assays to detect the high antibody concentrations during the acute phase of HEV infection.

The seroprevalence found with assay B is in line with data from a study conducted with >4000 sera from a representative sample of the German general population [9]. However, many HEV seroprevalence studies performed in industrialized countries show significant differences [8]. Rates range from 0.3% (for assay A) in Greece [10] to 52.5% (for assay B) in an HEV-hyperendemic region in southwest France [11]. Our results suggest that different HEV antibody detection assays strongly affect these seroprevalence estimates. This is in line with data from 2 recently published studies. Bendall et al analyzed 500 sera from blood donors in the United Kingdom by means of assays A and B [8]. The authors reported a seroprevalence of 3.6% by assay A, compared with 16.2% by assay B, which is surprisingly similar to the figures we found. Moreover, a literature review by the same authors showed that low

HEV prevalences (<5%) in developed countries have mostly been obtained with insensitive assays [8]. In this context, Mansuy et al repeated a study on the anti-HEV IgG seroprevalence among approximately 500 blood donors in southwestern France [11]. The authors reported that 52.5% of sera tested positive by assay B, suggesting that HEV is hyperendemic in the Midi-Pyrénées region. Interestingly, in a previous study from the same region, the authors used assay A and found that only 16.6% of specimens were positive [12]. These discrepancies demonstrate the need for validation of antibody assays against sera from individuals with proven HEV genotype 3 infection. Only a few conclusive validation studies have been published so far [8, 13].

In contrast to the high HEV seroprevalence found in our study, there was a surprisingly low incidence of hepatitis E in Germany in 2010 (0.3 cases/100 000). A likely explanation is that most autochthonous HEV infections are either asymptomatic or unrecognized [14].

In conclusion, our study shows that differences in the sensitivities of anti-HEV IgG tests must be taken into account when interpreting published HEV seroprevalences, because estimates differ significantly, depending on the assay used.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

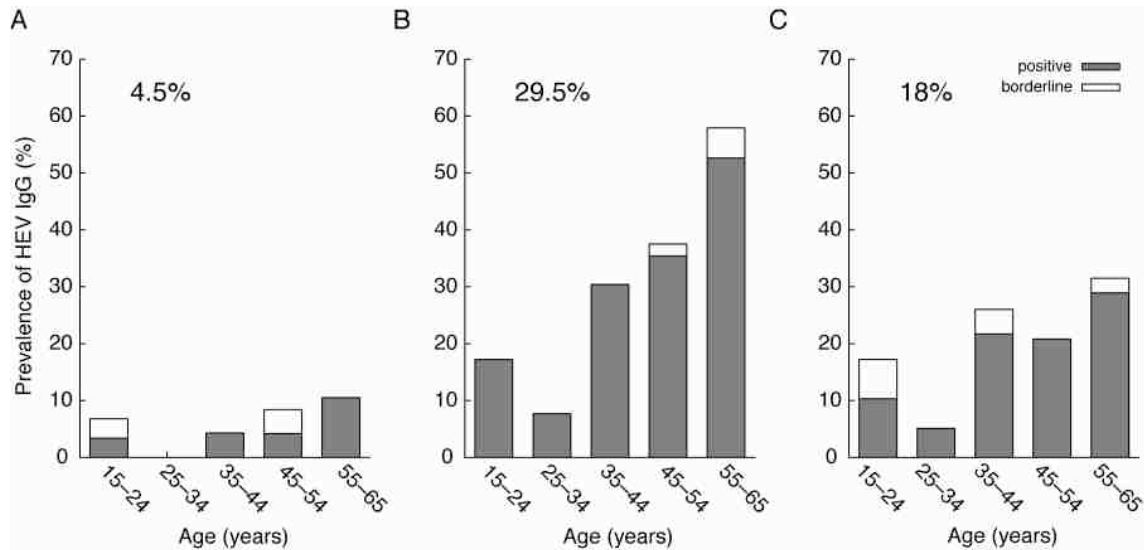
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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## SUPPLEMENTARY DATA

**Supplementary Figure 1.**



**Supplementary Figure 1.** Prevalence of hepatitis E virus (HEV) IgG by age group in 200 sera of individuals from southeastern Germany, 2010. Percentages in the inset indicate total seroprevalences (positive results) for all samples. Sera were analyzed with the MP Diagnostics HEV IgG ELISA (A), Axiom Diagnostic HEV IgG EIA (B) and Mikrogen *recom*Line HEV IgG (C). Filled bars represent positive, open bars borderline results.

**Supplementary Table 2. Assay results for HEV IgG in healthy individuals and in patients with acute hepatitis E<sup>a</sup>**

HEV IgG ELISA (MP Diagnostics) (A)	HEV IgG ELISA (Axiom Diagnostic) (B)	<i>RecomLine</i> HEV IgG (Mikrogen) (C)	Healthy individuals	Patients with acute hepatitis E
–	–	–	132	0
–	+	–	22	1
–	~	–	3	0
–	+	+	28	2
+	+	+	7	25
+	+	~	1	0
~	+	+	1	2
+	–	–	1	0
~	–	–	1	0
~	–	~	1	0
–	–	~	3	0
Total			200	30

<sup>a</sup> –, negative; +, positive; ~, borderline test result.

HEV IgG assay result combinations (columns 1–3) are shown for healthy individuals (column 4) and in acute hepatitis E cases (column 5). For example, the first line below the heading shows, that concordantly negative test results (–) were found with assays A–C in 132 healthy individuals, whereas this combination was never observed in acute hepatitis E cases. Accordingly, line 5 shows that positive results (+) were found with tests A–C in 7 healthy individuals, whereas this combination was observed in 25 acute hepatitis E cases. In line 6, 1 sample from an healthy individual was positive in assays A and B, but borderline (~) with assay C. The latter combination was not observed in acute cases.

**Supplementary Table 3. Patients with acute hepatitis E virus infection<sup>a</sup>**

Pat. No.	Sex	Age (yrs)	Geographic location (country, state)	Year of diagnosis	Material analyzed (PCR)	HEV geno-type	Virus concn (cp/ml)
1	M	61	DE, Saxony	2007	Serum	3	pos.
2	M	28	IN	2007	Serum	1	pos.
3	F	42	DE, Lower Saxony	2007	Serum	3	pos.
4	M	42	DE, Saxony	2007	Serum	3	$5.1 \times 10^7$
5	M	75	DE, Saxony	2007	Serum	3	$5.1 \times 10^5$
6	M	30	DE, Saxony	2007	Serum	n.a.	$1.6 \times 10^4$
7	M	39	DE, Baden-Württemberg	2008	Serum	3	$1.9 \times 10^3$
8	F	63	DE, Schleswig-Holstein	2008	Serum	n.a.	$<10^4$
9	M	53	DE, Saxony	2009	Serum	n.a.	$1.9 \times 10^4$
10	M	58	DE, Baden-Württemberg	2009	Serum	3	$3.3 \times 10^5$
11	M	26	DE, Northrhine-Westfalia	2009	Serum	3	$<10^4$
12	M	41	DE, Bavaria	2009	Stool	3	$<10^4$
13	F	55	DE, Thuringia	2010	Serum	3	pos.
14	M	12	IN or PK	2010	Serum	1	$2.4 \times 10^6$
15	M	50	DE, Baden-Württemberg	2010	Serum	3	$<10^4$
16	M	57	DE, Bavaria	2010	Serum	3	$4.4 \times 10^5$
17	F	49	DE, Brandenburg	2010	Serum	3	$<10^4$
18	M	34	DE, Brandenburg	2010	Plasma	3	$<10^4$
19	M	n.a.	DE, Mecklenburg-Vorpommern	2010	Serum	3	$<10^4$
20	F	17	PK	2010	Serum	1	$2.9 \times 10^6$
21	M	43	DE, Hamburg	2010	Stool	3	$<10^4$
22	M	52	DE, Northrhine-Westfalia	2010	Serum	3	$<10^4$
23	M	68	DE, Northrhine-Westfalia	2010	Serum	n.a.	$<10^4$

24	M	71	DE, Northrhine-Westfalia	2010	Serum	3	$1.6 \times 10^6$
25	M	37	DE, Bavaria	2010	Serum	3	$6.3 \times 10^4$
26	M	50	DE, Brandenburg	2010	Serum	3	$1.4 \times 10^4$
27	F	39	DE, Rhineland-Palatinate	2010	Serum	3	$2.6 \times 10^5$
28	F	35	DE, Rhineland-Palatinate	2010	Serum	3	$<10^4$
29	M	41	DE, Saxony-Anhalt	2010	Plasma	3	$4.8 \times 10^4$
30	M	52	DE, Schleswig-Holstein	2010	Serum	3	$2.6 \times 10^6$

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<sup>a</sup> Pat, patient; M, male; F, female; DE, Germany; IN, India; PK, Pakistan; cp, copies; n.a., not available; pos., positive. All sera were positive in at least one anti-HEV IgM assay.