

# Serologic Assays Specific to Immunoglobulin M Antibodies against Hepatitis E Virus: Pangenotypic Evaluation of Performances

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**Six immunoassays for detecting immunoglobulin M antibodies to hepatitis E virus were evaluated. Serum samples representing acute infection by each of the 4 viral genotypes as well as nonacute hepatitis E virus infection constituted the test panels. Diagnostic sensitivities and specificities as well as interassay agreement varied widely. Analytical sensitivity limits also were determined and were found to be particularly disparate.**

Classic hepatitis E virus (HEV) infection, which manifests as waterborne outbreaks of jaundice, is associated with transmission of HEV belonging to genotypes 1 and 2. Nonimported HEV infection that is encountered in industrialized countries is linked to indigenous transmission of HEV strains belonging to genotypes 3 and 4 [1]. In the United States, clinically manifested HEV infection is seldom reported, although HEV infection appears widespread [2], a disparity that may be due to low-dose exposure to HEV, asymptomatic infection by relatively avirulent HEV strains, or missed or underdiagnosed HEV infection. Diagnostic assays have been developed for detection of anti-HEV immunoglobulin M (IgM), but evaluations of their performances were based on analyzing serum samples obtained from case patients who were infected by  $\leq 2$  HEV genotypes [3, 4]. It would be desirable, in clinical practice and for disease surveillance, to rely on assays that identify incident HEV infection regardless of the genotypic background of the infecting strain. Here, we report findings

from an evaluation of assays that included serum samples from patients infected by HEV representing each of the 4 genotypes.

**Materials and methods.** Six immunoassays, named I–VI, were evaluated. All adopted the indirect (sandwich) format. Assay I used, as antigen, a recombinant protein expressed in baculovirus from amino acid positions 112–606 of open reading frame 2 of the Sar-55 HEV strain (genotype 1) (provided by R. Purcell) [5]. Assay II used, as antigens, proteins expressed in *Escherichia coli* from positions 452–617 in open reading frame 2 originating from the HEV-Morocco (genotype 1), Mexico-14 (genotype 2), US-1 (genotype 3), and China-9829 (genotype 4) strains [6]. Assay III was purchased from International Immuno-Diagnostics (no. 287), assay IV from MP Biomedicals (no. 21162–096), assay V from Diagnostics Systems (no. E-152), and assay VI from Mikrogen (no. 5005). Study samples were tested once by each of the 6 anti-HEV-IgM assays.

Fifty serum samples that fulfilled the following criteria constituted the diagnostic sensitivity panel: (1) origin from acutely jaundiced patients in whom acute hepatitis A, B, and C virus infection and chronic hepatitis B and C virus infection were serologically excluded; (2) presence of HEV RNA as detected by reverse-transcription polymerase chain reaction (RT-PCR); and (3) definitive assignment of the HEV genotype after nucleotide sequencing. Their provenance, year of col-

Received 15 February 2010; accepted 12 April 2010; electronically published 25 June 2010.

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**Clinical Infectious Diseases** 2010;51(3):e24–e27

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1058-4838/2010/5103-00E24\$15.00  
DOI: 10.1086/654801

lection, HEV genotype carried, and sample size, respectively, are as follows: Turkmenistan (1985 and 1994, genotype 1,  $n = 5$ ), Chad (2004, genotype 1,  $n = 10$ ), Mexico (1985–1986, genotypes 2 and 3,  $n = 4$  and  $n = 1$ , respectively), United States (2004–2008, genotype 3,  $n = 4$ ), Hungary (2001–2006, genotype 3,  $n = 9$ ), and China (2005–2006, genotype 4,  $n = 17$ ). The Turkmenistan, Chad, Mexico, and US samples [7–9] were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta from 1985 through 2008. Before dispatch to the CDC for this study, samples from Hungary were initially referred to the Regional Laboratory for Virology in Pécs [10], and those from China were initially referred to the Second Hospital and the Southeast University School of Medicine in Nanjing [11]. All serum samples in this panel and in the specificity panel (see below) were stripped of personal identifiers before being tested at CDC. Survey protocols were approved by CDC's Institutional Review Board.

To constitute the specificity panel, 229 serum samples were assembled, originating from anti-HEV-immunoglobulin G (IgG)-seropositive household contacts of HEV-infected patients in Chad [8] and Mexico [9] who were not jaundiced at the time of interview but who reported being jaundiced  $>6$  months earlier ( $n = 31$ ); US patients with acute hepatitis A ( $n = 35$ ), acute hepatitis B ( $n = 35$ ), and acute hepatitis C ( $n = 28$ ) infection who were identified from 1996 through 2006 in CDC's Sentinel Counties Study of Acute Viral Hepatitis [12]; and US blood donations ( $n = 100$ ) purchased from Vital Products. Samples found to be reactive in any 1 assay were tested for HEV RNA by RT-PCR [7].

A quantitation standard, pool 6, which contains anti-HEV IgM at a concentration of 860 Walter Reed (WR) units/mL [3], was serially diluted. Each dilution was tested once in assays III–VI. A positive control, pool 7 (which contains anti-HEV IgM at an estimated concentration of 195–304 WR units/mL) [3], was tested undiluted. Pools 6 and 7 were provided by R. Jarman and K. Myint. A human anti-HEV serum, preparation 95/584, which contains trace amounts of anti-HEV IgM [3, 4], was purchased from the National Institute for Biological Standards and Control and was tested undiluted.

**Results.** Fifty-three samples in the specificity panel were reactive in  $\geq 1$  assay. One sample in the acute hepatitis C virus infection group was found to be positive for HEV RNA (arrows, Figure 1A) and was characterized to carry genotype 3. Accordingly, it was considered to be a constituent of the sensitivity panel. The sensitivity panel therefore comprised 51 serum samples, and the specificity panel comprised 228 samples.

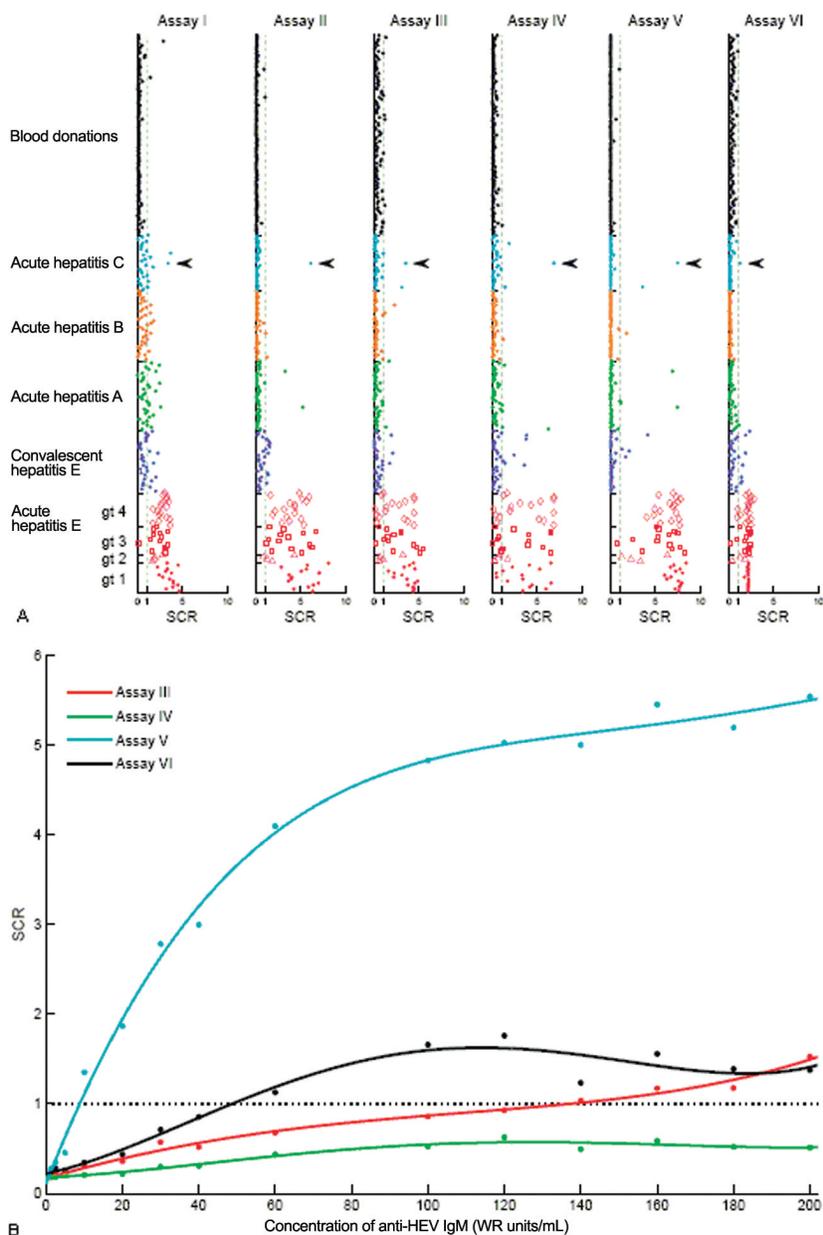
The distribution of signal-to-cut-off ratios is shown in Figure 1A. For each assay, the interpanel difference in the mean signal-to-cut-off ratios was highly significant ( $P < .001$ , Mann-Whitney test) and remained so after exclusion of convalescent hepatitis E samples from the specificity panel.

Individual assay sensitivities and specificities, respectively, were as follows: assay I, 98% (95% confidence interval [CI], 88%–99.9%) and 78.2% (95% CI, 72.1%–83.2%); assay II, 98% (95% CI, 88%–99.9%) and 93% (95% CI, 88.7%–95.8%); assay III, 82% (95% CI, 68.1%–90.9%) and 91.2% (95% CI, 86.6%–94.4%); assay IV, 72% (95% CI, 57.2%–83.3%) and 93% (95% CI, 88.2%–95.5%); assay V, 98% (95% CI, 88%–99.9%) and 95.2% (95% CI, 91.3%–97.4%); and assay VI, 92% (95% CI, 79.9%–97.4%) and 95.6% (95% CI, 91.9%–97.8%).  $\kappa$  coefficients ranged 0.46–0.8 (mean, 0.53), with the highest value attained between assays II and V and between assays V and VI.

Analytical response curves generated by assays III–VI after application to dilutions of pool 6 are shown in Figure 1B. The detection limit of assay III was estimated at 137 WR units/mL, the detection limit of assay V was estimated at 9 WR units/mL, and the detection limit of assay VI was estimated at 49 WR units/mL. Pool 7 was reactive in all 4 assays. The sensitivity limit of assay IV therefore lay between the lowest dilution of pool 6 and the upper limit of anti-HEV IgM estimated in pool 7. Preparation 95/584 was reactive only in assay V.

**Discussion.** A pangenotypic approach to the evaluation was adopted to identify assays that would accurately diagnose acute HEV infection, whether imported to or autochthonous in the United States and Europe. In-house assays were assessed as candidate confirmatory assays, but the emphasis of the study was placed on evaluating commercial assays currently in use. One commercial assay (assay V) achieved the same sensitivity value as the 2 in-house assays, which was the highest sensitivity value. The lower sensitivity values achieved by assays III and IV mainly reflect their lesser ability to detect anti-HEV IgM in the genotype 2, genotype 3, and genotype 4 groups (Figure 1A). The order of diagnostic sensitivity values observed for the commercial assays (from highest to lowest: assay V, assay VI, assay III, and assay IV) matches the order observed after analytic sensitivity measurements that used pool 6 (Figure 1B).

Reactivities in the specificity panel, particularly those in the convalescent HEV group, may reflect the true presence of anti-HEV IgM. These samples were included in the study to control for the effect of competition between IgM and IgG for antigen binding that can occur in indirect assays [4]. For each assay, the difference between the signal-to-cut-off ratios generated in the sensitivity panels and those generated in the specificity panels was highly significant, regardless of whether that group was included, so the effect of competition in reducing sensitivity was considered to be minimal. The serum sample in the specificity panel that was initially included in the acute hepatitis C virus infection group likely originated from a patient with chronic hepatitis C virus infection who was undergoing HEV superinfection. The present case definition of acute hepatitis C virus infection is based serologically on elevated alanine aminotransferase activity, the presence of anti-hepatitis C virus



**Figure 1.** Quantitative assessments of sensitivity and specificity of 6 anti-hepatitis E virus (HEV)-immunoglobulin M assays. *A*, Scatterplots showing distribution of signal-to-cut-off ratios (SCRs) generated in serum samples that initially constituted the acute HEV infection panel (displayed as red symbols, each symbol denoting a specific HEV genotype: circle, genotype 1; triangle, genotype 2; square, genotype 3; and rhombus, genotype 4) and the nonacute HEV infection panel (as circles in colors other than red). Arrows indicate the single serum sample in the acute hepatitis C virus infection group found to be reactive in all 6 assays and subsequently characterized to carry HEV genotype 3. *B*, Graph of analytical response curves generated from the application of assays III, IV, V, and VI to indicated dilutions of pool 6. Approximation was performed with polynomials of degree 4 (for assay III,  $R^2 = 0.989$ ; for assay IV,  $R^2 = 0.966$ ; for assay V,  $R^2 = 0.995$ ; and for assay VI,  $R^2 = 0.964$ ). The dotted horizontal line denotes an SCR value of 1. WR, Walter Reed.

IgG, and the absence of IgM antibodies against hepatitis A and B viruses; acute HEV infection is not specifically excluded [13]. The findings obtained from this sample point to the potential role of HEV testing in refining the case definition of acute hepatitis C infection.

There are several limitations to this study. First, for diagnostic

sensitivity determination, samples from patients in the non-viremic, late-acute phase of infection were excluded, thus precluding evaluation of the assays' ability to detect decreasing anti-HEV-IgM levels. Second, serum samples from anicteric patients were also excluded, so that test samples would be associated with primary HEV infection but not with secondary

infection, during which IgM may be elicited anamnesticly [3]. Third, because of sample-volume limitations and to evaluate a broad array of assays, panel serum samples and pool 6 dilutions were assayed only once, which precluded setting confidence limits to observation values and assessments of assay precision. Fourth, the number of genotype-2 samples was small, reflecting their rarity.

This evaluation revealed appreciable variability in sensitivity, specificity, and interassay agreement among anti-HEV-IgM immunoassays. For the accurate diagnosis of acute HEV infection, the use of assays validated to have the best performance characteristics is recommended.

### Acknowledgments

We are grateful to K. Foytich, N.-T. Le, and D. Chen, for assistance with assay performance, and R. Purcell, R. Jarman, and K. Myint, for providing reagents.

**Financial support.** CDC.

**Potential conflicts of interest.** All authors: no conflicts.

### References

1. Teo CG. Much meat, much malady: changing perceptions of the epidemiology of hepatitis E. *Clin Microbiol Infect* **2010**; 16:24–32.
2. Kuniholm MH, Purcell RH, McQuillan GM, Engle RE, Wasley A, Nelson KE. Epidemiology of hepatitis E virus in the United States: results from the third National Health and Nutrition Examination Survey, 1988–1994. *J Infect Dis* **2009**; 200:48–56.
3. Seriwatana J, Shrestha MP, Scott RM, et al. Clinical and epidemiological relevance of quantitating hepatitis E virus-specific immunoglobulin M. *Clin Diagn Lab Immunol* **2002**; 9:1072–1078.
4. Yu C, Engle RE, Bryan JP, Emerson SU, Purcell RH. Detection of immunoglobulin M antibodies to hepatitis E virus by class capture enzyme immunoassay. *Clin Diagn Lab Immunol* **2003**; 10:579–586.
5. Bryan JP, Tsarev SA, Iqbal M, et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J Infect Dis* **1994**; 170:517–521.
6. Obriadina A, Meng JH, Ulanova T, et al. A new enzyme immunoassay for the detection of antibody to hepatitis E virus. *J Gastroenterol Hepatol* **2002**; 17(suppl 3):S360–S364.
7. Albetkova A, Drobeniuc J, Yashina T, et al. Characterization of hepatitis E virus from outbreak and sporadic cases in Turkmenistan. *J Med Virol* **2007**; 79:1696–1702.
8. Nicand E, Armstrong GL, Enouf V, et al. Genetic heterogeneity of hepatitis E virus in Darfur, Sudan, and neighboring Chad. *J Med Virol* **2005**; 77:519–521.
9. Velázquez O, Stedler HC, Avila C, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986–1987. *JAMA* **1990**; 263:3281–3285.
10. Reuter G, Fodor D, Forgach P, Katai A, Szucs G. Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *J Clin Virol* **2009**; 44:277–281.
11. Dong C, Dai X, J Shao, Hu K, Meng J. Identification of genetic diversity of hepatitis E virus (HEV) and determination of the seroprevalence of HEV in eastern China. *Arch Virol* **2007**; 152:739–746.
12. Nainan OV, Armstrong GL, Han XH, Williams I, Bell BP, Margolis HS. Hepatitis A molecular epidemiology in the United States, 1996–1997: sources of infection and implications of vaccination policy. *J Infect Dis* **2005**; 191:957–963.
13. Daniels D, Grytdal S, Wasley A. Surveillance for acute viral hepatitis—United States, 2007. *MMWR Surveill Summ* **2009**; 58:1–27.