Received: 26 July 2016 | Revised: 26 October 2016 | Accepted: 2 December 2016

DOI 10.1002/jmv.24741

RESEARCH ARTICLE

WILEY JOURNAL OF MEDICAL VIROLOGY

Variability in the performance characteristics of IgG anti-HEV assays and its impact on reliability of seroprevalence rates of hepatitis E

Maja Kodani | Nourine A. Kamili | Alexandra Tejada-Strop | Amanda Poe | Maxine M. Denniston | Jan Drobeniuc | Saleem Kamili

Division of Viral Hepatitis, National Center for HIV, Hepatitis, STDs, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Correspondence
Maja Kodani, PhD, Division of Viral Hepatitis, National Center for HIV, Hepatitis, STDs, and TB Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop A33, Atlanta, GA 30333.
Email: mkodani@cdc.gov

Hepatitis E is a major public health problem in developing countries and is increasingly being recognized as a cause of substantial sporadic viral hepatitis infections in industrialized countries. Variable rates of hepatitis E seroprevalence have been reported from the same geographic regions depending on the assay used. In this study, we evaluated the performance characteristics of four assays which included two commercial assays, Wantai HEV-IgG ELISA kit (Wantai, China), and DS-EIA-ANTI-HEV-G kit (DSI, Italy), one NIH-developed immunoassay (NIH-55 K, Kuniholm et al. [2009] Journal of Infectious Diseases 200:48-56), previously used in several major seroprevalence studies and one in-house Western blot assay (CDC-WB). The limit of detection of IgG anti-HEV is 100 mIU/mL for Wantai assay, 200 mIU/mL for CDC-WB assay, 1000 mIU/mL for DSI assay, and 40 mIU/mL for NIH-55 K assay. Pairwise concordance between the four assays ranged from 56% to 87%. The concordance among all four assays was observed in 52% of the samples, while the concordance among three assays was observed in 37% of the samples. These data show a wide discordance between various IgG anti-HEV assays and warrant a comprehensive evaluation of all the assays using well characterized global serum reference panels.

KEYWORDS
enzyme assays, hepatitis E virus, IgG anti-HEV, protein electrophoresis, seroprevalence

1 | INTRODUCTION

Hepatitis E Virus (HEV) is the sole member of the family Hepeviridae which is divided into two genera: Orthohepevirus (mammalian and avian HEV) and Piscihepevirus (trout HEV). Orthohepevirus A includes all human HEV isolates consisting of two genotypes isolated from humans alone (HEV-1 and 2) and three genotypes isolated in both humans and various animal species and associated with the potential zoonotic cases (HEV-3, 4, and 7).[1–6] HEV is a small, non-enveloped single-stranded, positive-sense RNA virus, 27-34 nm in diameter, and a genome of approximately 7200 nucleotides.[7,8] It expresses three open reading frames (ORF), ORF1, ORF2, and ORF3[9]; ORF1 encodes the non-structural proteins, ORF2 encodes the capsid protein, and the small ORF3 protein is a cytoskeleton-associated phosphoprotein having regulatory functions. HEV genotype 1 is endemic to many developing countries in Asia and Africa, with the infection varying from sub-clinical to fulminant hepatitis especially in pregnant women in whom mortality rates as high as 30% have been reported.[10] HEV genotype 3 infections are prevalent in many developed countries, and can lead to chronicity in solid organ transplant recipients and immunocompromised patients.[10]

Anti-HEV seroprevalence rates ranging from 15.9% to 23% have been reported from various selected populations in the United States.[11] An assessment of hepatitis E seroprevalence in the United States was conducted through the Third National Health and Nutrition Examination Survey (NHANES-III), based on testing of 18 695 sera using an NIH-developed enzyme immunoassay (EIA) based on the ORF2-encoded 55 kDa protein, henceforth referred to as NIH-55 K assay.[12] This study showed a prevalence of 21% in the general non-institutionalized U.S. population sampled from 1988 through 1994.[12] In a recent study, based on testing a 6000-member subset of the same NHANES-III (1988-1994) samples by a commercially available assay,

DS-EIA-ANTI-HEV-G (DSI-EIA, DSI, Germany), a seroprevalence rate of 10.2% was reported.[13] In the same study and using the same assay, a seroprevalence rate of 6% was observed, when 7885 sera samples from NHANES-IV (2009-2010) participants were tested.[13] Variable seroprevalence rates in the same populations in the same geographic regions have previously been reported when different assays were applied.[14–17]

In this study, we tested a subset of NHANES-III serum samples using a commercially available kit, Wantai HEV-IgG ELISA henceforth referred to as Wantai-EIA (Wantai, China). All samples were also tested by our newly developed in-house IgG anti-HEV Western blot assay, henceforth referred to as CDC-WB assay. IgG anti-HEV results of the samples previously tested by NIH-55 K assay[12] were included in the analysis. This study further highlights the lack of reliability of IgG anti-HEV assays in generating accurate seroprevalence rates and warrants a need for comprehensive evaluation of these assays using well characterized global reference panels.

2 | MATERIALS AND METHODS

2.1 | Study samples

Specimens for this study were selected from a repository of NHANES-III (1988-1994) serum samples that were previously tested for IgG anti-HEV by NIH-55 K assay showing 21% seroprevalence.[12] A stratified random sample of 6000 of these specimens were retested by a commercially available assay, the DSI-EIA, and the results were reported in our recent study.[13] From this sample set, previously tested by DSI-EIA and NIH-55 K, we selected a total of 1804 specimens for further testing by Wantai-EIA and the CDC-WB assay. This included a subset of samples positive by both assays ($n = 757$), negative by both assays ($n = 244$), and all discordant samples between the two assays (DSI-EIA positive/NIH-5 K negative [$n = 31$]; DSI-EIA negative/NIH-55 K positive [$n = 772$]).

We also evaluated the analytical and diagnostic sensitivity of the assays by testing serial dilutions of the WHO Reference Reagent for Hepatitis E Virus Antibody (95/584) (National Institute for Biological Standards and Control, United Kingdom) and seven seroconversion panels which included one panel of serial samples collected from an imported case of hepatitis E genotype 4[18] and six commercially available HEV genotype 3 seroconversion panels (DiaMex GmbH, Germany). These seroconversion panels were tested for IgM anti-HEV and HEV RNA by DiaMex GmBH using the recomWell assays (Mikrogen Diagnostik, Germany).

2.2 | Serological testing

All samples were tested for IgG anti-HEV by two commercially available assays, the DS-EIA previously[13] and Wantai HEV-IgG EIA in this study, according to the manufacturers' instructions. The calculations for the signal to cut-off ratio (SCR) were performed based on the manufacturer's recommendations for both assays. In addition, all samples were also tested by an in-house Western blot assay, the CDC-WB assay. Briefly, the CDC-WB assay used an antigen (p166) based on amino acids 452-617 of ORF2 of HEV genotype 3.[19] Precast 12% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) were loaded with 80 µL of the purified p166 antigen and transferred to a nitrocellulose membrane using the iBlot Gel Transfer Apparatus (Novex, by Life Technologies) and standard procedures. Serum samples, prepared by diluting 60 µL of each sample in 550 µL of dilution buffer (5% nonfat dry milk, 0.05 M Tris-buffered saline, 0.05% Tween-20) were loaded on the Mini-PROTEAN II Multiscreen Apparatus (Bio-Rad) and incubated for 12-16 h at room temperature on a rocking platform and washed with dilution buffer three times for 10 min, each. This was followed by incubation of the samples with peroxidase labeled anti-human IgG (gamma) antibody (KPL Inc., Gaithersburg, MD), diluted 1:1000 in dilution buffer, for 90 min at room temperature, then washing of the membranes and their development using 3,3′-diaminobenzidine (Sigma-Aldrich Co. LLC).

3 | RESULTS

3.1 | Analytic and diagnostic sensitivity of anti-HEV IgG assays

The limit of detection (LOD) of the three methods, Wantai EIA, CDC-WB, DSI-EIA assay was 100, 200, and 1000 mIU/mL, respectively (Fig. 1). Using the same WHO Reference Reagent for Hepatitis E Virus Antibody (95/584) standard, the LOD of IgG anti-HEV for NIH-55 K has been previously reported as 40 mIU/mL.[12] The diagnostic

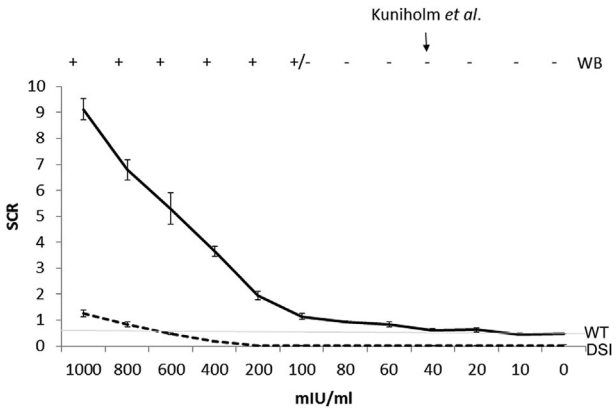

FIGURE 1 Limit of detection. Limit of detection (LOD) was determined for the CDC-WB assay, DSI EIA, and Wantai EIA, using serial dilutions of the WHO reference reagent for hepatitis E virus antibody (95/584). The CDC-WB positivity is denoted by + or − at the top, the results of the DSI EIA (DSI) are shown in dotted line, and the results of the Wantai EIA (WT) are shown in solid line. Light gray line denotes the positive/negative signal to cut-off ratio. The most sensitive assay was Wantai EIA at 100 mIU/mL followed by the CDC-WB assay at 200 mIU/mL and DSI EIA at 1000 mIU/mL. All experiments were performed in triplicate, and the brackets represent one standard deviation

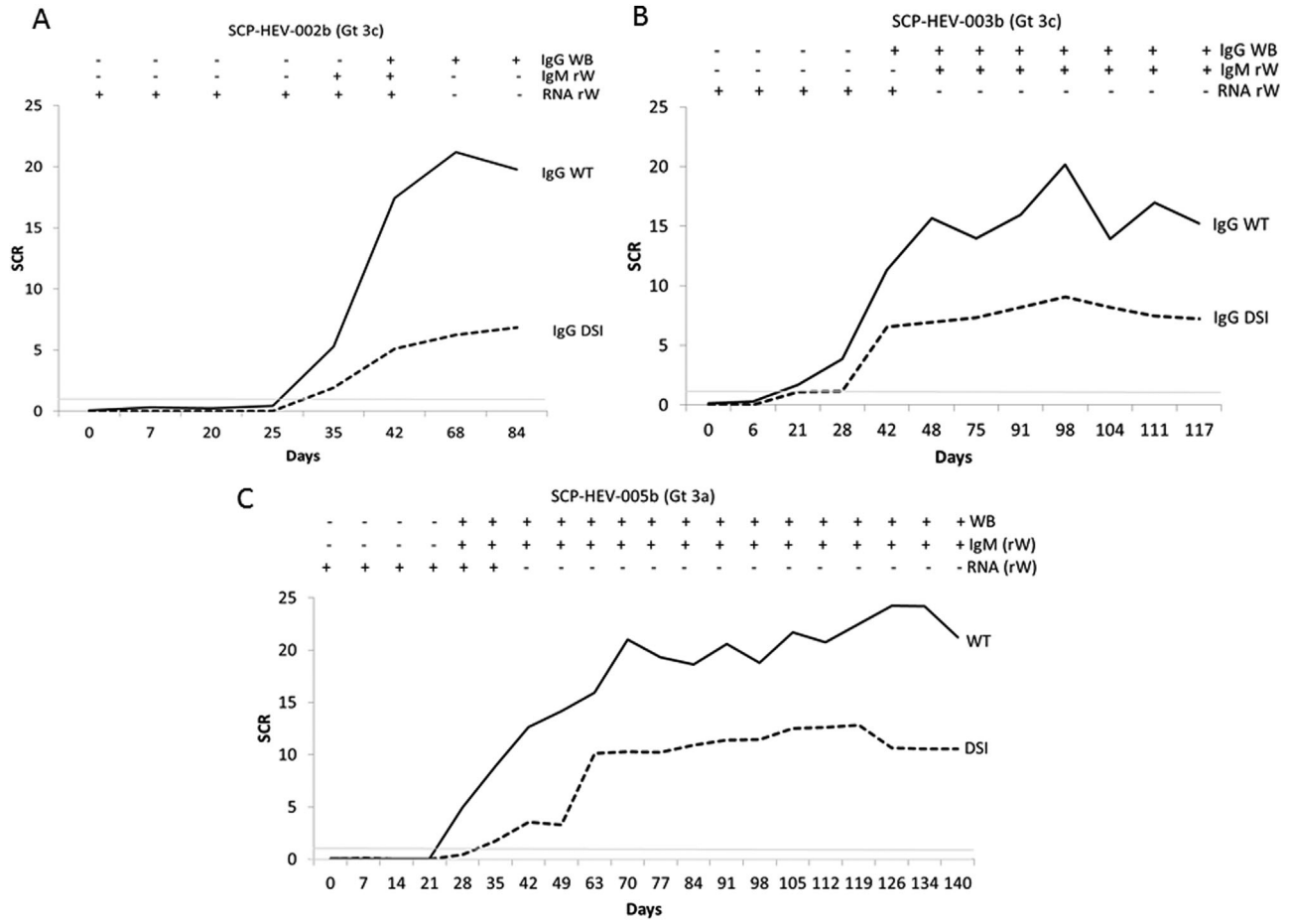

FIGURE 2 Seroconversion panels from hepatitis E patients. Six patient seroconversion panels were evaluated by all three assays, including the CDC-WB, DSI EIA, and Wantai EIA. CDC-WB positivity is denoted by + or − at the top, the results of the DSI EIA (DSI) are shown in dotted line, and the results of the Wantai EIA (WT) are shown in solid line. Light gray line denotes the positive/negative signal to cut-off ratio. Commercially-available IgM anti-HEV and HEV RNA results determined by the recomWell kits (rW) were included for comparison. Days on the x-axis represent the number of days since the original blood draw, denoted by 0 days. HEV seroconversion panels from DiaMex, SCP-HEV-002b, genotype 3c (Panel A), SCP-HEV-003b from DiaMex, genotype 3c (Panel B), and SCP-HEV-005b, genotype 3a (Panel C), are shown as representative examples

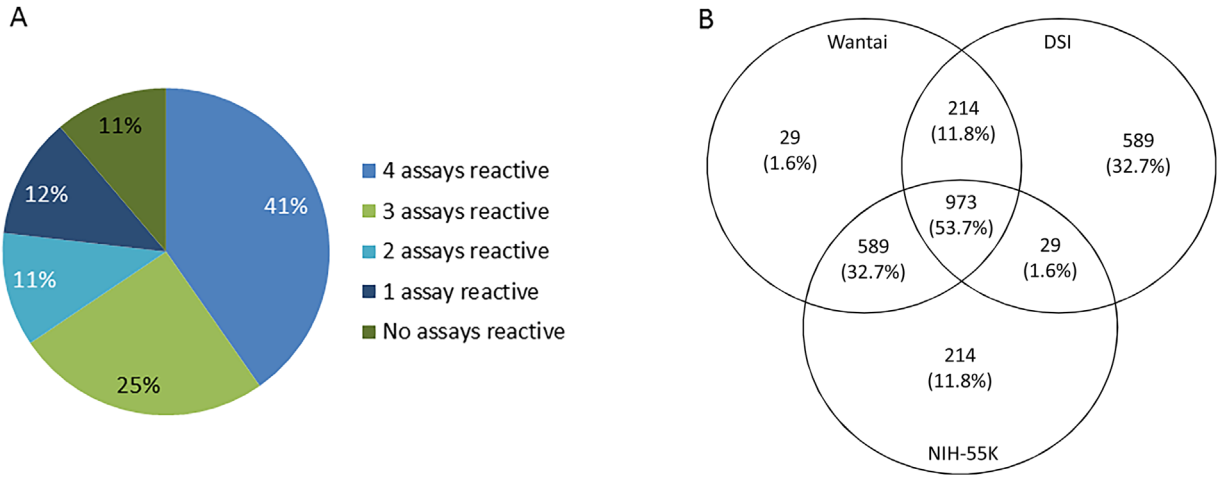

FIGURE 3 Assay concordance distribution. (A) Pie graph representation of the percentages of concordant and partially discordant assays, including all four assays, the CDC-WB, DSI EIA, Wantai EIA, and NIH-55K.[12] (B) Venn diagram of EIA assay concordance showing the number of specimens in each category as well as the percentage of the total number of 1804 specimens

sensitivity of the three assays, determined by testing sequential samples from HEV infected patients, showed that Wantai assay detected IgG anti-HEV approximately a week earlier than the other two assays (Fig. 2). IgG anti-HEV was detectable by all three assays in all the subsequent samples during the acute and convalescent phases of HEV infection. Analysis of the SCR showed that the Wantai EIA generates higher SCR values than the DSI EIA. The seroconversion panels were not tested by NIH-55 K assay due to lack of its availability.

3.2 | IgG anti-HEV concordance

Out of 1804 samples tested in this study, 1529 samples (85%) were positive by NIH-55 K EIA,[12] 1354 samples (75%) were positive by Wantai EIA, 1227 samples (68%) were positive by CDC-WB, and 788 samples (44%) were positive by the DSI EIA. Of these 1804 samples tested by three assays (DSI-EIA, Wantai EIA, and CDC-WB), in this study and by NIH-55 K elsewhere[12] 727 (41%) were IgG anti-HEV positive and 202 (11%) were negative in all four assays (Fig. 3), resulting in 52% of the analyzed samples being concordant by all four assays. In addition, 456 (25%) were positive in three of the assays, 203 (11%) in two assays, and 216 (12%) in one assay (Fig. 3). Overall comparison of the three EIAs revealed that of the total of 1804 samples, 1588 (88%) were positive by at least two different assays, while 12% were positive by only one assay (Fig. 3).

Scatterplots with pairwise comparison of individual EIAs showed that even in the specimens positive by two assays, there was only moderate positive correlation between SCRs (Fig. 4). The correlation coefficients ranged from 0.518 to 0.607, with significant *P* values (Fig. 4).

Pairwise concordance between assays, including both positive and negative samples, ranged from 55.5% to 86.5% (Table 1). Wantai EIA exhibited higher concordance with CDC-WB and NIH-55 K assay, but lower concordance with the DSI EIA. In addition, specificity and sensitivity were calculated using 1804 specimen data, using each of the four assays as a reference assay. The results of this analysis are shown in Table 2.

4 | DISCUSSION

The aim of the current study was to determine the analytic and diagnostic sensitivity and inter-assay concordance of four IgG anti-HEV assays, some used in a number of published studies and some currently in use in various diagnostic laboratories worldwide. Of the two commercially available assays used in this study, Wantai assay had the highest analytic sensitivity with a limit of detection of 100 mIU/mL, which also contributed to its better diagnostic sensitivity detecting IgG anti-HEV, approximately a week earlier than any of the other assays used in this study. While the overall diagnostic sensitivity of the two commercially available assays and one in-house Western blot assay, determined by testing six seroconversion panels, was excellent and concordant, there was wide variability in their detection rates, when samples from a healthy general population were tested. A

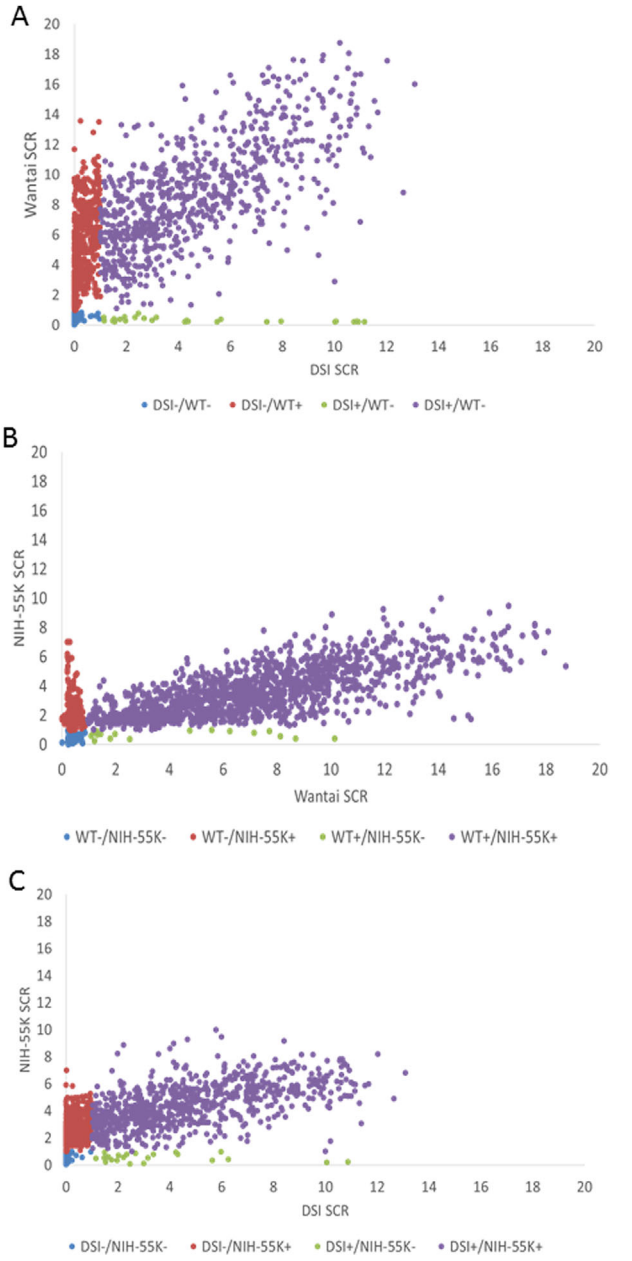

FIGURE 4 Graphic comparison of assay disparity. (A) Pairwise comparison plot of the signal to cut-off ratios (SCR) for the DSI EIA and Wantai EIA (r2 = 0.523, $P < 0.005$). Purple dots represent specimens positive by both assays, blue dots represent specimens negative by both assays, red dots represent DSI EIA negative-Wantai EIA positive specimens, and green dots represent DSI EIA positive-Wantai EIA negativespecimens. (B) Pairwise comparison plot of the SCR for the NIH-55K[12] data and Wantai EIA (r2 = 0.607, $P < 0.005$). Purple dots represent specimens positive by both assays, blue dots represent specimens negative by both assays, red dots represent NIH-55K positive-Wantai EIA negative specimens, and green dots represent NIH-55K negative-Wantai EIA positive specimens. (C) Pairwise comparison plot of the SCR for the NIH-55K data and DSI EIA (r2 = 0.518, $P < 0.005$). Purple dots represent specimens positive by both assays, blue dots represent specimens negative by both assays, red dots represent NIH-55K positive-DSI EIA negative specimens, and green dots represent NIH-55K negative-DSI EIA positive specimens

TABLE 1 Concordance between pairs of assays, showing the percentage of both concordant (bold font) and discordant data points (regular font)

	Discordant results (%)			
Concordant results (%)	Western blot	DSI ELISA	Wantai ELISA	NIH-55K {Kuniholm, 2009 #639}
Western blot		27.2	14.6	22.7
DSI ELISA	**72.8**		34.3	44.5
Wantai ELISA	**85.4**	**65.7**		13.5
NIH-55K {Kuniholm, 2009 #639}	**77.3**	**55.5**	**86.5**	

seroprevalence rate of 21% was previously reported for the NHANES-III—1988-1994 cycle using the NIH-55 K assay, which was reported to have a detection limit of 40 mIU/mL.[12] We previously tested 6000 samples stratified by select demographic characteristics with the DSI-EIA[13] to estimate anti-HEV IgG prevalence and found that precision was not comparable to estimates of Kuniholm et al.[12] Using the DSI-EIA, we reported a seroprevalence of 10.5%, and observed a decline in seroprevalence (6%) when the NHANES-IV (2009-2010 cycles) samples were tested.[13]

Four assays compared in this study used recombinant antigens from ORF2 region of the HEV genome, with the main difference being the size of the recombinant antigen. In addition, the DSI EIA contained a fusion protein with additional antigenic regions from ORF2 and ORF3. The inter-assay concordance of the four assays, two non-commercial, NIH-55 K and CDC-WB, and two commercial assays, DSI-EIA and Wantai assay, was determined using a subset of the samples from NHANES-III population. As expected, NIH-55 K assay had the highest detection rate of IgG anti-HEV positive samples (N = 1529, 85%) followed by Wantai assay (N = 1354, 75%), CDC-WB (N = 1227, 68%), and DSI-EIA assays (N = 788, 44%). The concordance among the four assays ranged from 56% to 87%. The issue of determining the performance characteristics of IgG anti-HEV assays and inter-assay concordance has been addressed in several studies, using samples from various populations. In one of the earliest such studies, overall pairwise assay concordance ranged from 41% to 89%, when twelve different assays were used to test the same sample panel.[20] The same study reported pairwise assay concordance among positive samples between 0% and 89%. A number of studies have previously addressed the issue of anti-HEV IgG assay disparity,[14–16,21–30] but these studies were limited in the number of assays used,[14,16,17,23,25–27,29] the number and nature of specimens evaluated,[16,21,25,29] lack of direct sample-by-sample comparison,[16,22,24,26,27,29] or exclusive evaluation of select populations.[7,16,17,21,25,28] Two of these studies performed direct comparison of Wantai EIA and DSI EIA showing that the sensitivity of these two assay was comparable, yet there were striking differences in concordance among the results.[7,15] Neither of these studies tested samples from a general population, but instead they tested seroconverted patients with previously confirmed acute HEV infection or immunocompromised and immunocompetent patients that were anti-HEV IgG positive. Our study provides a sample-by-sample comparison of almost 2000 samples, obtained from a general population, each evaluated by four different assays, at different times, and analyzed in parallel.

Testing of all samples with our in-house Western blot assay, CDC-WB, showed its highest concordance with Wantai EIA, followed by NIH-55 K and DSI-EIA (Table 1). We used the CDC-WB assay as a reference assay to determine the specificity and sensitivity of the other three assays (Table 2). In addition, we observed a direct correlation of sensitivity of the assays with their limit of detection; in pairwise comparisons between three EIAs, the majority of discordant specimens (96%) were detected by the assay with the lowest LOD (Fig. 4). However, in every pairwise comparison (Fig. 4), a smaller proportion of specimens were positive by the assay with the highest LOD. Furthermore, the SCR values obtained for the samples positive by the assay with the highest LOD represented strong positive results, suggesting that there may be some differences in antigen recognition between assays. The data from this study are consistent with several previous studies that showed the Wantai EIA as the most sensitive assay for detection of IgG anti-HEV.[16,23,27,29,30] However,

TABLE 2 Performance of assays using each assay as a reference point and others as comparators. Ninety-fifth percentile confidence intervals are listed in the brackets

	Reference assay			
Specificity (%)*/sensitivity (%)*	Western blot	DSI ELISA	Wantai ELISA	NIH-55K {Kuniholm, 2009 #639}
Western blot		54.2 [51.2-57.3]/96.7 [95.5-98.0]	84.9 [81.6-88.2]/85.6 [83.7-87.5]	80.4 [75.7-85.1]/ 76.7 [74.6-78.8]
DSI ELISA	95.5 [93.8-97.2]/62.1 [59.4-64.8]		94.2 [92.1-96.4]/56.3 [53.6-58.9]	88.7 [85.0-92.5]/ 49.5 [47.0-52.0]
Wantai ELISA	66.2 [62.3-70.1]/94.6 [93.2-95.7]	41.7 [38.7-44.8]/96.7 [95.5-98.0]		87.6 [83.8-91.5]/ 86.3 [84.6-88.1]
NIH-55K {Kuniholm, 2009 #639}	38.3 [34.3-42.3]/95.6 [94.5-96.8]	24.0 [21.4-26.6]/96.1 [94.7-97.4]	53.6 [49.0-58.2]/97.5 [96.7-98.3]	

*[95% Confidence Interval]

the issue of specificity of this and other commercially available assays remains to be determined.

The disparity between the assays for detection of anti-HEV IgG is evident from this study and has been reported in several previous studies; however, this study included not only samples from well characterized clinical hepatitis E cases, but also a large number of samples from the healthy general population. The variable performance of commercial anti-HEV assays, and a wide discordance between them, warrants a comprehensive evaluation of these assays using well characterized global reference panels which will help in the validation of reliable assays and subsequent determination of accurate seroprevalence of hepatitis E worldwide.

ACKNOWLEDGMENTS

We thank Dr. Chen Dong, formerly a visiting scientist in our laboratory, for preparation and purification of p166 antigen, and Natasha Khudyakov for helping with serology.

CONFLICTS OF INTEREST

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centres for Disease Control and Prevention.

REFERENCES

1. Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, Heyries L, Raoult D, Gerolami R. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis*. 2010;202: 825–834.
2. Lee GH, Tan BH, Chi-Yuan Teo E, Lim SG, Dan YY, Wee A, Kim Aw PP, Zhu Y, Hibberd ML, Tan CK, Purdy MA, Teo CG. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150: 355–357.
3. Miyashita K, Kang JH, Saga A, Takahashi K, Shimamura T, Yasumoto A, Fukushima H, Sogabe S, Konishi K, Uchida T, Fujinaga A, Matsui T, Sakurai Y, Tsuji K, Maguchi H, Taniguchi M, Abe N, Fazle Akbar SM, Arai M, Mishiro S. Three cases of acute or fulminant hepatitis E caused by ingestion of pork meat and entrails in Hokkaido, Japan: zoonotic food-borne transmission of hepatitis E virus and public health concerns. *Hepatol Res*. 2012;42:870–878.
4. Teo CG. Much meat, much malady: changing perceptions of the epidemiology of hepatitis E. *Clin Microbiol Infect*. 2010;16:24–32.
5. Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, Koenig M, Jameel S, Harrison TJ, Meng XJ, Okamoto H, van der Poel WH, Purdy MA. Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol*. 2016;97:537–542. DOI: 10.1099/jgv.0.000393
6. Smith DB, Simmonds P. International committee on taxonomy of viruses hepeviridae study G, Jameel S, Emerson SU, Harrison TJ, Meng XJ, Okamoto H, Van der Poel WH, Purdy MA. Consensus proposals for classification of the family hepeviridae. *J Gen Virol*. 2014;95: 2223–2232.
7. Aggarwal R, Jameel S. Hepatitis E. *Hepatology*. 2011;54:2218–2226.
8. Holla RP, Ahmad I, Ahmad Z, Jameel S. Molecular Virology of Hepatitis E virus. *Semin Liver Dis*. 2013;33:3–14.
9. Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology*. 1991;185:120–131.
10. Khuroo MS, Khuroo MS, Khuroo NS. Hepatitis E: discovery, global impact, control and cure. *World J Gastroenterol*. 2016;22: 7030–7045.
11. Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, Purcell RH. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. *J Clin Microbiol*. 1997;35:1244–1247.
12. 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.
13. Teshale EH, Denniston MM, Drobeniuc J, Kamili S, Chong-Gee T, Holmberg SD. Decline in hepatitis E virus antibody prevalence in the United States from 1988-1994 to 2009-2010. *J Infect Dis*. 2015;211:366–373. DOI: 10.1093/infdis/jiu466
14. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a Re-Evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol*. 2010;82:799–805.
15. Pas SD, de Man RA, Mulders C, Balk AH, van Hal PT, Weimar W, Koopmans MP, Osterhaus AD, van der Eijk AA. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerg Infect Dis*. 2012;18:869–872.
16. Rossi-Tamisier M, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. *J Clin Virol*. 2013;56:62–64.
17. Wu WC, Su CW, Yang JY, Lin SF, Chen JY, Wu JC. Application of serologic assays for diagnosing acute hepatitis E in national surveillance of a nonendemic area. *J Med Virol* 2014;86:720–728.
18. Drobeniuc J, Greene-Montfort T, Le NT, Mixson-Hayden TR, Ganova-Raeva L, Dong C, Novak RT, Sharapov UM, Tohme RA, Teshale E, Kamili S, Teo CG. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005-2012. *Emerg Infect Dis*. 2013;19: 218–222.
19. Dong C, Meng J, Dai X, Liang JH, Feagins AR, Meng XJ, Belfiore NM, Bradford C, Corn JL, Cray C, Glass GE, Gordon ML, Hesse RA, Montgomery DL, Nicholson WL, Pilny AA, Ramamoorthy S, Shaver DD, Drobeniuc J, Purdy MA, Fields HA, Kamili S, Teo CG. Restricted enzooticity of hepatitis E virus genotypes 1 to 4 in the United States. *J Clin Microbiol*. 2011;49:4164–4172.
20. Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E virus antibody Serum panel evaluation group. *Hepatology*. 1998;27: 857–861.
21. Abravanel F, Mansuy JM, Huynh A, Kamar N, Alric L, Peron JM, Recher C, Izopet J. Low risk of hepatitis e virus reactivation after haematopoietic stem cell transplantation. *J Clin Virol*. 2012;54: 152–155.
22. Abravanel F, Chapuy-Regaud S, Lhomme S, Dubois M, Peron JM, Alric L, Rostaing L, Kamar N, Izopet J. Performance of two commercial assays for detecting hepatitis e virus RNA in acute or chronic infections. *J Clin Microbiol*. 2013;51:1913–1916.
23. Abravanel F, Chapuy-Regaud S, Lhomme S, Miedouge M, Peron JM, Alric L, Rostaing L, Kamar N, Izopet J. Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients. *J Clin Virol*. 2013;58:624–628.
24. Avellon A, Morago L, Garcia-Galera del Carmen M, Munoz M, Echevarria JM. Comparative sensitivity of commercial tests for hepatitis E genotype 3 virus antibody detection. *J Med Virol*. 2015; 87:1934–1939.
25. Khan A, Tanaka Y, Kurbanov F, Elkady A, Abbas Z, Azam Z, Subhan A, Raza S, Hamid S, Jafri W, Shih J, Xia N, Takahashi K, Mishiro S, Mizokami M. Investigating an outbreak of acute viral hepatitis caused

by hepatitis E virus variants in Karachi, South Pakistan. *J Med Virol*. 2011;83:622–629.

26. Park HK, Jeong SH, Kim JW, Woo BH, Lee DH, Kim HY, Ahn S. Seroprevalence of anti-hepatitis E virus (HEV) in a Korean population: comparison of two commercial anti-HEV assays. *BMC Infect Dis*. 2012;12:142.
27. Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF, Osterhaus AD, van der Eijk AA. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. *J Clin Virol*. 2013;58:629–634.
28. Traore KA, Rouamba H, Nebie Y, Sanou M, Traore AS, Barro N, Roques P. Seroprevalence of fecal-oral transmitted hepatitis A and E virus antibodies in burkina faso. *PLoS ONE*. 2012;7:e48125.
29. Vernier M, Rossi-Tamisier M, Richet H, Brouqui P, Parola P, Bouamri Y, Colson P, Gautret P. Anti-hepatitis E virus antibody prevalence in French expatriate workers. *Int J Infect Dis*. 2013;17:e1082–e1084.
30. Wenzel JJ, Preiss J, Schemmerer M, Huber B, Jilg W. Test performance characteristics of anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. *J Infect Dis*. 2013;207:497–500.

How to cite this article: Kodani M, Kamili NA, Tejada-Strop A, et al. Variability in the performance characteristics of IgG anti-HEV assays and its impact on reliability of seroprevalence rates of hepatitis E. *J Med Virol*. 2017;89:1055–1061. https://doi.org/10.1002/jmv.24741