Disparities in detection of antibodies against hepatitis E virus in US blood donor samples using commercial assays

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Abstract

Background—Reported hepatitis E virus (HEV) antibody assay performance characteristics are variable. Using a subset of surplus US blood donation samples, we compared assays for detecting anti-HEV IgM and IgG or total anti-HEV antibodies.

Methods—Samples from 5040 random blood donations, all HEV-RNA negative, collected primarily in the Midwestern US in 2015 were tested for anti-HEV IgM and IgG or total anti-HEV using assays manufactured by Diagnostic Systems, Wantai and MP Biomedicals.

Results—Overall, percent detection for anti-HEV IgG and total anti-HEV was 11.4%, and for anti-HEV IgM was 1.8%. Nine samples were reactive for anti-HEV IgM by all assays giving a recent infection rate of 0.18%. Anti-HEV IgG/total anti-HEV detection rates increased with age. Inter-assay agreement was higher among the IgG anti-HEV/total anti-HEV assays (84%) than the IgM assays (22%). Regression analyses of signal-to-cutoff ratios from IgG/total antibody assay were heteroskedastic indicating no constant variance among these assays suggesting they may detect different epitopes, or were affected by waning or less avid antibodies in the US donor population.

Conclusions—Although similar percentages of IgG anti-HEV/total anti-HEV detection were observed across the three commercial assays, each assay detected a unique sample subpopulation and were heteroskedastic when compared pairwise. Discordance was higher among anti-HEV IgM assays, but a recent HEV infection rate of at least 0.18% was estimated based on assay concordance.

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Keywords

Donors; Transfusion-transmitted Disease - Hepatitis; Infectious Disease Testing

Introduction

Hepatitis E is caused by the hepatitis E virus (HEV)[1]. HEV is a member of the *Hepeviridae* family. Variants that infect humans belong to the species *Orthohepevirus A*[2,3]. This species has seven recognized genotypes of which 5, genotypes 1, 2, 3, 4 and 7 are known to infect humans[3,4]. Although acute hepatitis E is usually self-limiting with low mortality (about 1–3%) the mortality rate can reach levels of up to 30% in pregnant women during the second and third trimester, who are infected with genotypes 1 or 2.[5,6]. HEV genotypes 3 and 4 are frequently associated with chronic infection in immunocompromised individuals, most notably solid organ transplant recipients leading to chronic hepatitis, cirrhosis and liver failure[7–9]. Early epidemiological studies indicated the virus was restricted to developing countries where it was transmitted fecal-orally through contaminated water causing large outbreaks. Sporadic cases of hepatitis E seen in developed countries were originally thought to be associated only with travel to endemic areas. However, more recent studies have shown that autochthonous hepatitis E, genotypes 3 and 4, can be found in developed countries transmitted zoonotically from infected animals through the consumption of raw or undercooked meat and offal[10–12]. Most cases of acute hepatitis E in developed countries tend to be asymptomatic[13–15]. Because asymptomatic HEV infection in blood donors has been documented via HEV RNA detection, and HEV has been documented to be transfusion transmitted, there is increasing concern worldwide about blood safety[15,16].

Antibody prevalence in blood donors varies depending on geographic location and the assays used. For example, in Europe anti-HEV IgG seroprevalence in blood donors ranges from 1.3% to 52%[14], increases with age, and higher IgG positivity are usually seen in males compared to females[17,18]. A study in the Netherlands using a single assay showed that IgG anti-HEV prevalence had decreased from 47% in 1988 to 21% in 2011[18], and in the US, the National Health and Nutrition Examination Survey (NHANES) using the same anti-HEV IgG assay found decreases in IgG prevalence from 10.2% during 1988–1994 to 6% during 2009–2010[19] . An analysis of 1939 US blood donors at the National Institutes of Health (NIH) in 2013 found IgG prevalence of 18.8% and IgM prevalence of 0.4%. IgG prevalence increased with donor age, with prevalence decreasing from 21.8% in 2006 to 16% in 2012. The NIH study used an in-house assay for the first analysis and a commercial assay for the subsequent analysis. None of the NIH donors were HEV-RNA-positive[20]. A subsequent study of 4499 HEV-RNA-negative samples at the American Red Cross (ARC), which was a subset of 18,829 donation samples collected in 2013 from which HEV RNA occurred in 1 per 9500 donations (95% confidence interval [CI], 1:2850–1:56,180), the antibody detection rates using a different assay was 7.7% for IgG and 0.58% for IgM[21]. Similarly, antibody prevalence increased with age, and was highest in the Midwest US (12.5%; odds ratio of 2.23 versus other US regions).

In spite of documented declines in HEV seroprevance, an increase in prevalence among 18- to 21-year old Dutch blood donors was observed[18]. There has also been an increase in the number of reported hepatitis E cases in several countries where it is a reportable disease[22–24]. However, it is not known whether this increase is due to increased HEV incidence or to increased reporting. Part of the problem is that clinical assays are validated to determine the status of an analyte in a symptomatic individual and not specifically for epidemiological studies of healthy populations. This is particularly true of HEV serological assays due to the lack of concordance has led to variable findings in asymptomatic populations when commercial assays have been compared[25–27]. Currently, there are no FDA-approved anti-HEV or HEV-RNA assays.

This study examined samples from 5040 US blood donations using three different commercial IgG and IgM anti-HEV assays to determine the anti-HEV prevalence rates and to examine the concordance among the immunoassays used.

Methods

Sample selection and preparation

Residual samples from blood donations made to the ARC from March 22 to April 3, 2015 were obtained. Samples from donations positive for routine disease markers (e.g., hepatitis B virus, hepatitis C virus and human immunodeficiency virus) were excluded. All samples used in this study were selected from approximately 50,000 samples screened by research-use only HEV RNA assays to exclude RNA positives (the results of HEV RNA screening are not considered in the current study). A total of 5040 random samples were enrolled. Blood was collected in plasma preparation tubes; the plasma from these tubes was stored at −70°C until tested[21]. Samples were tested under code with donor identities not available to investigators. Where appropriate, basic demographics were obtained from an ARC research database associated with this study. The study was approved by the ARC Institutional Review Board. As part of the donation consent, all donors were provided with an information sheet describing future potential uses of their surplus samples for studies on transfusion-transmissible infections. The samples were anonymized and sent to CDC for testing.

Antibody testing

This study used six enzyme immunoassays from three commercial companies: Diagnostic Systems Incorporated (DSI S.r.l., Milan, Italy (hereafter, DSI); MP Biomedicals Asia Pacific Pte. Ltd., Singapore (MP); and Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China (Wantai)). The detection of HEV IgM antibodies used three assays: DS-EIA-ANTI-HEV-M (batch: E-152, DSI), HEV IgM ELISA 3.0 (23162-096, MP) and HEV-IgM ELISA (WE-7192, Wantai). The detection of HEV IgG antibodies used two assays: DS-EIA-ANTI-HEV-G (E-151, DSI) and HEV-IgG ELISA (WE-7292, Wantai). The sixth assay detected total anti-HEV antibodies: HEV ELISA 4.0 (23542-096, MP). All assays were run according to the manufacturers' instructions, except for the Wantai assays for which initially positive samples were not retested, to standardize with the DSI and MP assays that do not require retesting. The Wantai IgG assay includes a gray zone outcome in addition to positive

and negative outcomes. A specific individual was designated to test assays from a specific vendor to minimize inter-operator error potentially introduced by sample or reagent handling.

The anti-HEV assays used in this study detect antibodies using different formats. For anti-HEV IgG detection, the DSI and Wantai assays use a recombinant capsid peptide to bind total antibody, then horseradish peroxidase (HRP)-conjugated anti-human IgG is used to detect captured IgG. MP uses a recombinant capsid peptide to capture total antibody and HRP-conjugated recombinant capsid peptide to bind to total bound antibody. For the detection of anti-HEV IgM, the DSI and the MP assays use a recombinant capsid peptide as the capture antigen to bind total antibody, and HRP-conjugated monoclonal mouse raised against anti-human IgM antibody to detect anti-IgM. The Wantai assay uses anti-μ antibody to capture total IgM and HRP-conjugated recombinant capsid peptide to bind to anti-HEV IgM.

Statistics

Pearson's Chi-squared test with Yates' continuity correction, 95% Confidence Intervals, odds ratios, regression analysis and Breusch-Pagan test for heteroskedasticity[28] were calculated in R (ver. 2.15.3)[29]. Heteroskedasticity indicates that the variation in a variable is unequal across the range of values of a second variable used to predict the first.

Results

Of the 5040 samples tested, 569 (11.29%; 95% CI, 10.43% to 12.20%; DSI) and 619 (12.28%; CI, 11.39% to 13.22%; Wantai) were reactive for anti-HEV IgG, and 537 (10.65%; CI, 9.82% to 11.54%; MP) were reactive for total anti-HEV antibody, yielding an average of 11.41% (Table 1). There was no significant difference between the numbers of reactive samples detected between IgG assays. Anti-HEV IgM testing resulted in 142 reactives (2.90%; CI, 2.45% to 3.40%: DSI), 93 (1.85%; CI, 1.49% to 2.26%: MP) and 34 (0.67%; CI, 0.47% to 0.94%; Wantai), yielding an average reactivity of 1.81% (Table 1). The extent of agreement between IgG anti-HEV/total anti-HEV and IgM anti-HEV assay sample detection is shown on Supplemental Table 1.

There was no statistical difference in the gender of the donors selected (male = 2682, female = 2358). The age range for donors was from 16 to 93 years of age (Figure 1). IgG anti-HEV detection rate increased with age, and all three assays exhibited similar uptrends (Figure 2, upper panel) with no significant differences seen among the trends. Age and gender adjustment of these data among the four states with the highest number of donors, Missouri, Kentucky, Illinois and Indiana, did not detect any appreciable differences in IgG anti-HEV detection rates by age group versus age and gender adjusted detection rates among the assays (Supplemental Figure 1). No trend in anti-HEV IgM seroprevalence with age was evident (Figure 2, lower panel).

Samples came from donors residing in 21 states. Differences by state of residence were analyzed in the five states with the highest number of donors; Missouri (MO, n=2080), Kentucky (KY, n=1120), Illinois (IL, n=887), Indiana (IN, n=813) and Kansas (KS, n=97).

All other states were excluded from the state by state comparison because of the low donor numbers, for example, the state with the next highest number of donations was California with 8 donors. Only anti-HEV IgG/total anti-HEV was analyzed because of the low number of anti-HEV-IgM positive samples by state. IgG anti-HEV/total anti-HEV prevalence percentages, as the mean among the three assays used to test specimens, across all assays within the five states having the highest numbers of donors ranged from Kansas having the lowest (5.15%) to Illinois and Missouri having the highest (12.70% and 14.01%, respectively) (Figure 3); although Kansas has the lowest prevalence, due to the low number of samples tested, its 95% confidence intervals overlaps those for Illinois and Missouri and thus are not significant (Table 2 and Supplemental Table 2).

Because this was a cross-sectional unlinked study, there is no way to estimate declines of anti-HEV over time, or when the donors were infected relative to the donation used in this study. However, looking at individuals with S/CO ratios above the cutoff for each assay allows the range of S/CO ratios for individuals within age ranges to be evaluated (Supplemental Figure 2). No significant difference or trend is observed in the range of S/CO ratios amongst these individuals by age group for any of the IgG/total anti-HEV assays used in this study.

Concordance between assays is shown in Figures 4A–C and Supplemental Table 1. Concordance among IgG anti-HEV/total anti-HEV -reactive specimens ranged from 443 samples reactive by all three assays, 454 to 499 samples reactive by two assays, and from 537 to 619 samples reactive by any given assay for an overall agreement of 84%. There was a reduction in prevalence of 11.41% (reactive by any assay) to 8.79% (reactive in all three assays). The highest agreement was between MP and Wantai (Figure 4A). For IgM, concordance ranged from 34 to 146 reactive by any given assay, 10 to 31 samples reactive by two assays, and for nine samples reactive by all three assays for an overall agreement of 22%. There was a reduction in prevalence from 1.81% (reactive by any assay) to 0.18% (reactive in all three assays). The highest agreement was between DSI and MP (Figure 4B). When concordance was compared between samples having both IgG anti-HEV /total anti-HEV and IgM anti-HEV reactivity, from 15 to 71 were reactive by the assays from any given manufacturer, and for seven samples were reactive by all six assays for an overall agreement of 40%, and a prevalence of 0.14%. The highest agreement was again between DSI and MP (Figure 4C). Nine samples had concordant IgM anti-HEV reactivity (including seven with concordant IgM/IgG reactivity), giving a frequency of 1 per 560 (0.18%; Figure 4B). Since IgM is a marker of recent infection that develops within 2 to 6 weeks following infection, these nine IgM-concordantly reactive donors were considered to be likely HEV infected recently. As Table 3 shows, the nine donors came from two states: Kentucky and Missouri; all except one 18-year old donor ranged in age from 47–81 years, and six were male.

Regression analysis (Figures 5A–C) was initially used to assess correlations among the assays. Pearson's product-moment correlation between the MP total antibody assay and the Wantai IgG assay was r(n=5038) = 0.89 (95% CI, 0.88–0.90), p < 0.00001, indicating strong correlation between these assays. However, this correlation was due to the large number of negative results (Fig 5C). If samples that are negative in both assays ("true negatives") were removed, the correlation for the remaining samples was r(n=497) = 0.74 (95% CI, 0.70–

0.80), p < 0.00001. Although this value could be considered to show reasonable agreement between these 2 assays, a plot of signal-to-cutoff (S/CO) ratios indicates that the variation between assays is heteroskedastic (Figures 5A–C; p <0.00001, using the Breusch-Pagan test for heteroskedasticity among all three IgG assays, and Supplemental Figure 3). An examination of the panels in Supplemental Figure 3 shows that no pair of assays has constant variation of data points around the regression line through the data. This result indicates that the null hypothesis of constant variance can be rejected between any two pairs of IgG assays, or, there is no relationship between S/CO ratios between pairs of assays. This outcome is similar if the DSI IgG assay was compared against either the MP or the Wantai assays (Figures 5A and 5B). The lack of constant variation is still seen when samples with saturated signal with the MP assay are removed (Supplemental Figure 3). The data suggest that each assay may be detecting an epitope not detectable by the other assays. This is further seen among the discordant samples, which are negative by one assay, but have a high S/CO by another assay (Figure 5, red and green data points). In some cases, the discrepancies are separated by more than 6 sigmas (Supplemental Figure 3).

Discussion

It has been estimated that there are 20 million HEV infections worldwide annually, leading to about 3.3 million symptomatic cases of hepatitis E and 56,600 HEV-related deaths[30,31]. In the US, an analysis of clinical cases of non A-C acute hepatitis from 2005 to 2012 found that 26 (17%) of 154 cases were due to HEV infection. There was a near-even split between travel-associated and autochthonous hepatitis E cases. The autochthonous cases were all infected by HEV genotype 3 and tended to occur among older patients compared to those with travel- associated hepatitis E[32].

The determination of anti-HEV serostatus remains enigmatic, and the results of various assays are often divergent. This is particularly true of healthy individuals like blood donors[27]. Recently pairwise concordance among three commercially available IgG anti-HEV assays and one laboratory developed assay ranged from 56 to 87% with a concordance of 52% observed in all samples tested among all four assays[26]. Another study of five assays for the detection of IgM and IgG found concordances of 71% and 70%. The limit of detection varied up to 19-fold for the IgM assays and 17-fold for the IgG assays[33]. A study of healthy US citizens using NHANES specimens found a decrease in IgG prevalence from 10.2% during 1988–1994 to 6% during 2009–2010 using the DSI assay[19]. IgG anti-HEV seroprevalence in NIH blood donors was 21.8% in 2006 with a decrease to 16.0% in 2012 using the Wantai assay[20], and 7.7% in ARC blood donors in 2013 using the MP assay[21]. The decreases in anti-HEV IgG rates observed in the NHANES and NIH studies are similar to observations seen in Germany where a study of 45 subjects found that anti-HEV IgG concentrations decreased significantly after 5 years[34], and a study of Dutch blood donors showed that IgG seroprevalence decreased from 19.8% in 1998 to 12.7% in 2011[18]. These last two studies observed seroreversion and HEV reinfections in some individuals despite pre-existing HEV antibodies.

This study examined the anti-HEV IgG/total anti-HEV and IgM anti-HEV detection percentages in US blood donors and the performance of three commercial assays for IgG/

total and IgM antibodies in this donor population. The assays used were from DSI, MP and Wantai. Overall the three assays yielded similar results. Within the total donor population, the IgG anti-HEV/total anti-HEV positivity was 11.41% (range by assay of 10.65% to 12.28%) and the IgM positivity was 1.81% (range by assay of 0.67% to 2.90%) (Table 1). An increase in anti-HEV IgG positivity was seen with increasing age, regardless of gender, as has been seen in other studies (Figure 2, top panel)[19–21]. However, no trend was seen for IgM seropositivity with increasing age (Figure 2, bottom panel). Differences were seen between genders, regardless of age, where more males than females were IgG reactive (Table 2). These results are in agreement with the earlier ARC study that only used the MP assay to test anti-HEV IgG[21]. However, in our study, the gender difference was significant in the total donor population with the DSI ($p < 0.005$) and Wantai ($p < 0.01$) assays, but not significant in the MP assay. When the five states with the most donors were compared individually, the differences between males and females were not significant except in Missouri, and only with the DSI assay ($p < 0.05$).

Agreement between the assays was better for the IgG/total anti-HEV than the IgM assays. The overall agreement among the IgG/total anti-HEV assays was 84% but was only 22% for the IgM assays (Figures 4A–C). Some of the poor agreement among the IgM assays appears to be due to the discordance between the DSI and MP assays (Fig. 4B). The difference between discordant and concordant results was 2 logs higher in the IgM assays than the IgG/total anti-HEV assays. The wide range in these values is due to the low concordance between the IgM assays (Figure 4B), which in turn is probably due to difference in the epitopes and assay formats used to detect IgM anti-HEV.

Despite the overall 84% agreement between the IgG/total antibody assays, the disparity in sample detection as well as S/CO ratios among discordant samples seen among all three assays could indicate that each assay is detecting an epitope(s) not detectable by the other assays (Figure 5, red and green data points). The presence of heteroskedasticity among the IgG/total assays indicates that the null hypothesis of constant variance should be rejected, further supporting the conclusion of differing epitope detection. Alternatively, these differences could be due in part to the varying formats used in these assays or waning or less avid antibodies in the donor population. Differences in detection rates between the IgG anti-HEV assays (DSI and Wantai) and the total antibody assay could be due to IgM-positive detection by the total antibody assay (the MP assay) not detected by the IgG-only assays. However, MP IgM anti-HEV positivity did not correlate with total antibody positivity. Detection of IgA-containing samples cannot be excluded, but there is no way to test for IgA anti-HEV. As has been previously suggested, these data indicate that a panel of well-characterized plasma samples from HEV-infected individuals needs to be created to validate anti-HEV assay performance. Additionally, some as yet undiscovered factor, as with protein Fv, may be interfering with detection of HEV epitopes[35]. Fv, a Fab binding factor, was found to interfere with an early in-house anti-HEV assay resulting in false-negative results.

The major limitations to this study include the fact that the assays use different antigens and detection formats with no established method to determine their absolute performance characteristics. Blood donors are a select low-risk population that does not mirror the general US population[36]. The assays used have been validated for clinical purposes in

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symptomatic persons but not for epidemiological studies in asymptomatic individuals. In addition, the performance of antibody assays is much better in patients with acute infection than in those with past infection[37]. Also, specimens initially positive by the Wantai assays were not retested as recommended in the manufacturer's instructions; however, one would expect a high correlation between initially reactive and repeatedly reactive samples in a commercial assay that has been widely used for anti-HEV studies worldwide. In addition, any potential reductions in IgG or IgM reactivity due to the absence of repeat testing are not expected to reduce the heteroskedasticity seen in the assays, nor to significantly alter the lack of concordance seen among the IgM assays.

In conclusion, our data indicate that these HEV IgG/total anti-HEV assays are useful for examining seroprevalence and associated trends in seroprevalence with an inter-assay agreement of 84%; however, the disagreement between these assays indicates that there is an associated discordance rate or difference in target detection driving variability as seen by the heteroskedasticity evident among the IgG assays. The case for the IgM assays is worse since no IgM assay evaluated here demonstrated clinical utility in the blood donor population tested, although an IgM response in HEV RNA-positive blood donors has been well characterized[38]. These data suggest that more reliable information on prevalence may be obtained from using concordant reactive results from multiple assays. Despite these limitations, and assuming that the IgM-positive period exceeds that for HEV RNA detection, we calculated a rate of recent infection of 0.18% (1:560) based on concordance among all three anti-HEV IgM assays, noting that none of the nine IgM-reactive samples was HEV-RNA positive (data not shown). A recent study in 20,000 US blood donors found low HEV-RNA positivity (0.01%) and thus a low burden of new infection in US blood donors[21].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

ARC	American Red Cross
DSI	Diagnostic Systems Incorporated
HEV	hepatitis E virus
HRP	horseradish peroxidase
MP	MP Biomedicals
NHANES	National Health and Nutrition Examination Survey

S/CO   signal-to-cutoff

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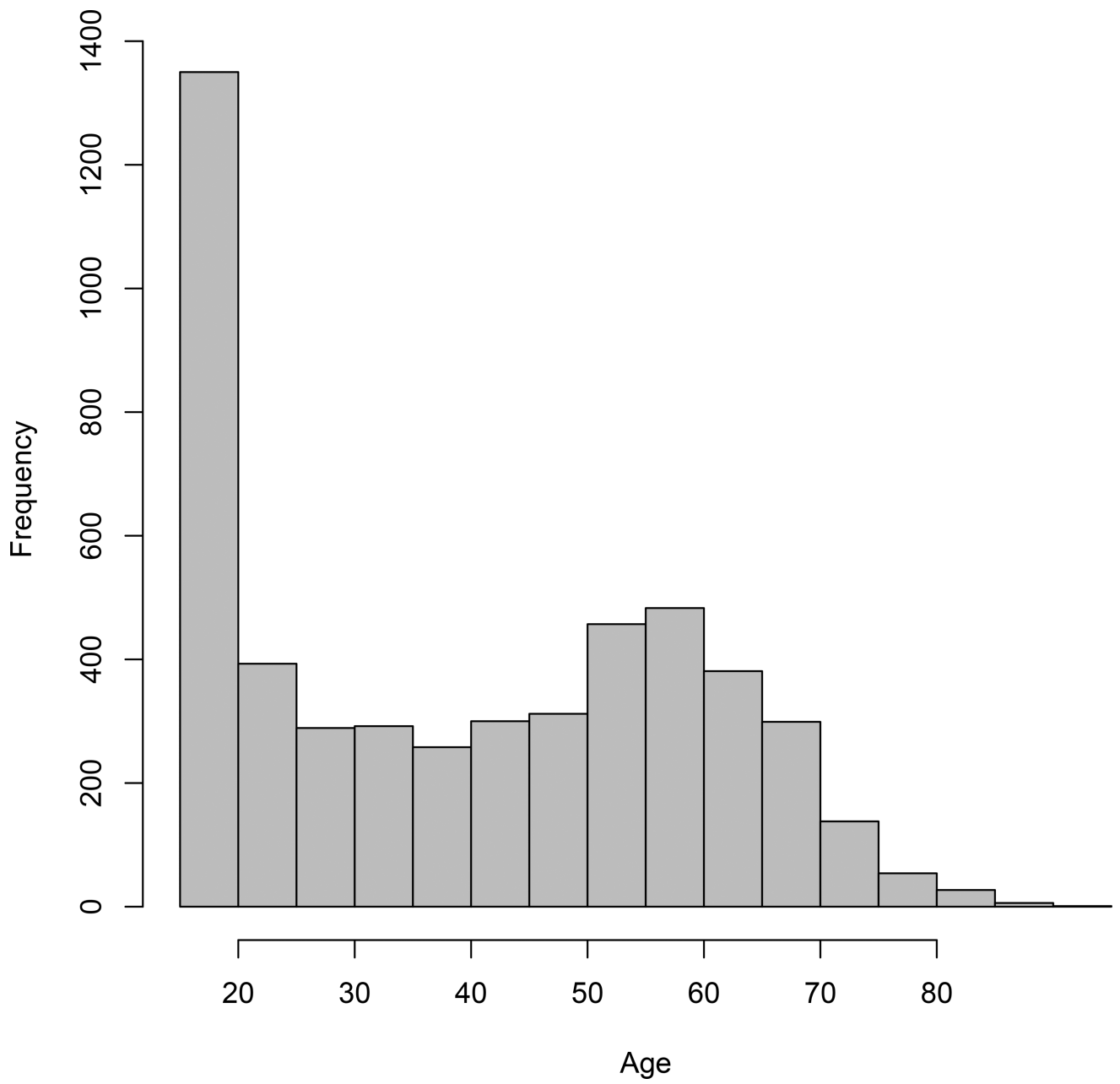
Figure 1.
Histogram of donor age distribution. Frequency distribution calculated using 5 year bins, age range (16 to 93).
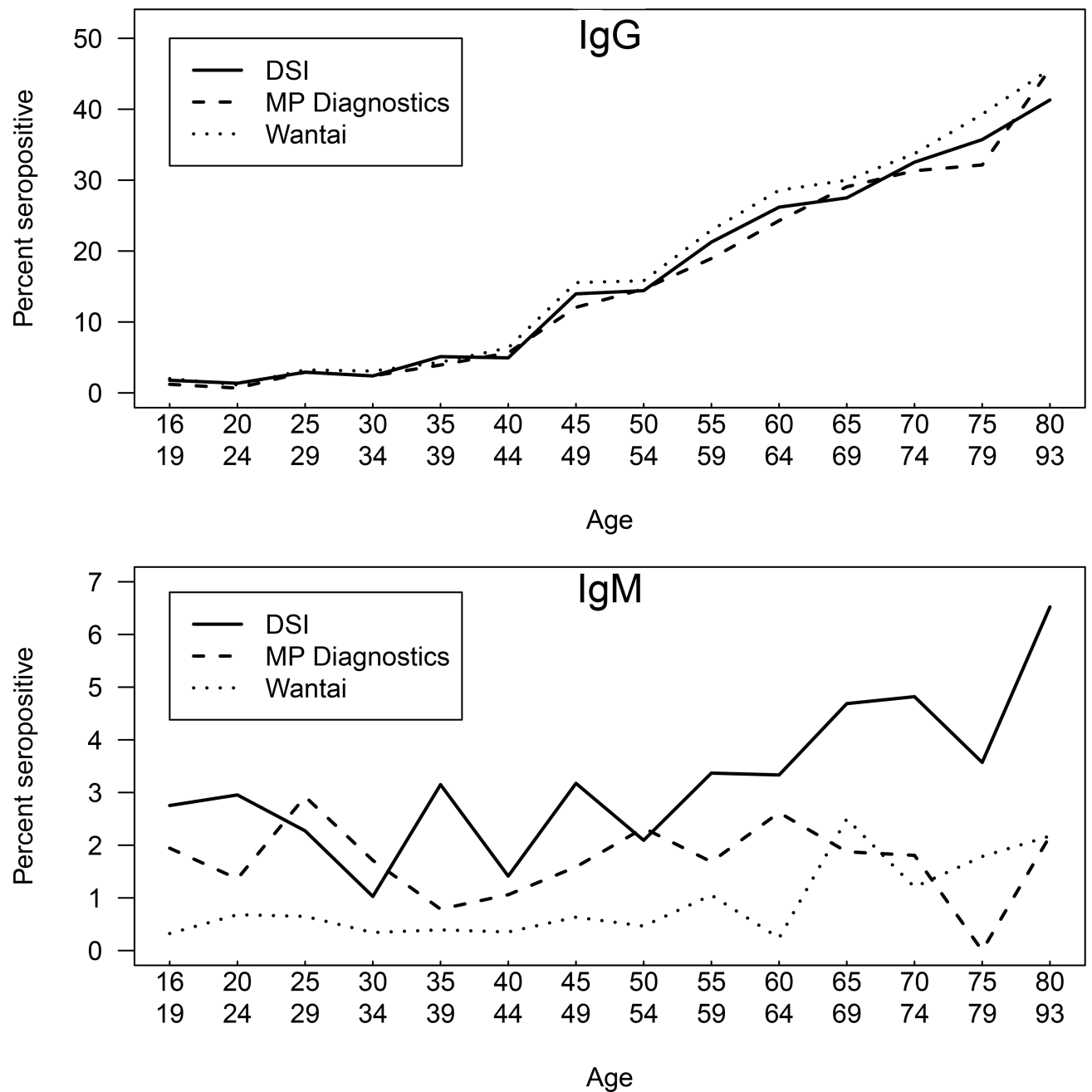
Figure 2.
Seroprevalence by age. Upper panel, anti-HEV IgG/total antibodies; lower panel, anti-HEV IgM. Solid line, DSI; dashed line MP Biomedicals; dotted line, Wantai. Data were plotted by averaging the seroprevalence by age range from donors 15 to 80 years of age in increments of 5 years, and all ages above 80 years of age formed the last range. Seroprevalence within each age range was plotted versus the mean age within the age range.
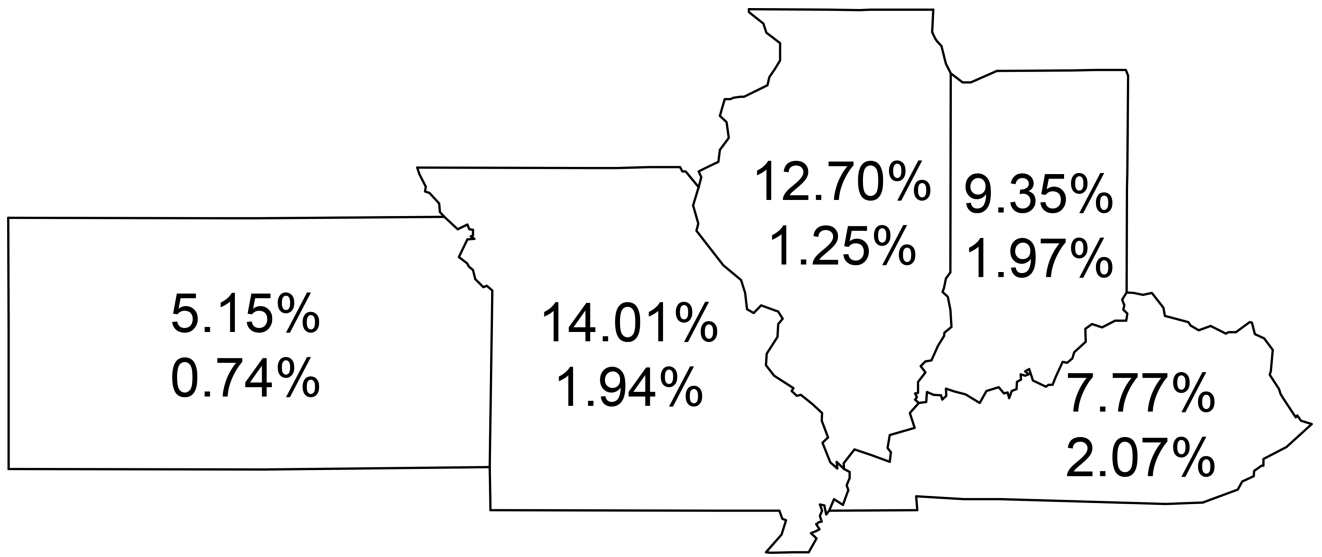
Figure 3.
Anti-HEV prevalence in the 5 states with the highest number of donors. The numbers within each state boundary are IgG/total antibody prevalence (upper number) and IgM prevalence (lower number). States from left to right: Kansas, Missouri, Illinois, Indiana and Kentucky.
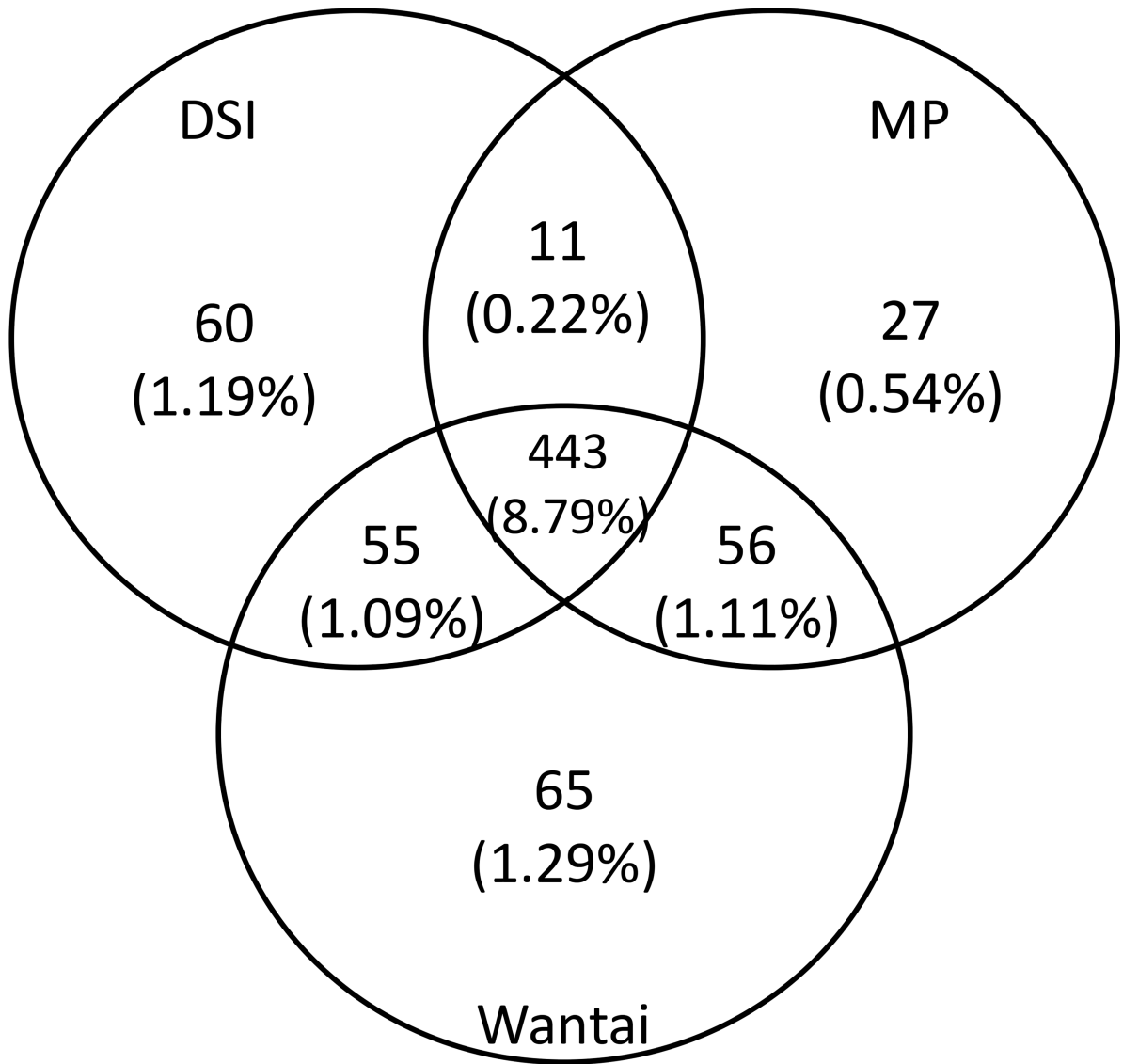
A. IgG

DSI

MP

11
(0.22%)

60
(1.19%)

27
(0.54%)

443
(8.79%)

55
(1.09%)

56
(1.11%)

65
(1.29%)

Wantai

B. IgM

DSI

MP

22
(0.44%)

106
(2.10%)

61
(1.21%)

9
(0.18%)

9
(0.18%)

1
(0.02%)

15
(0.30%)

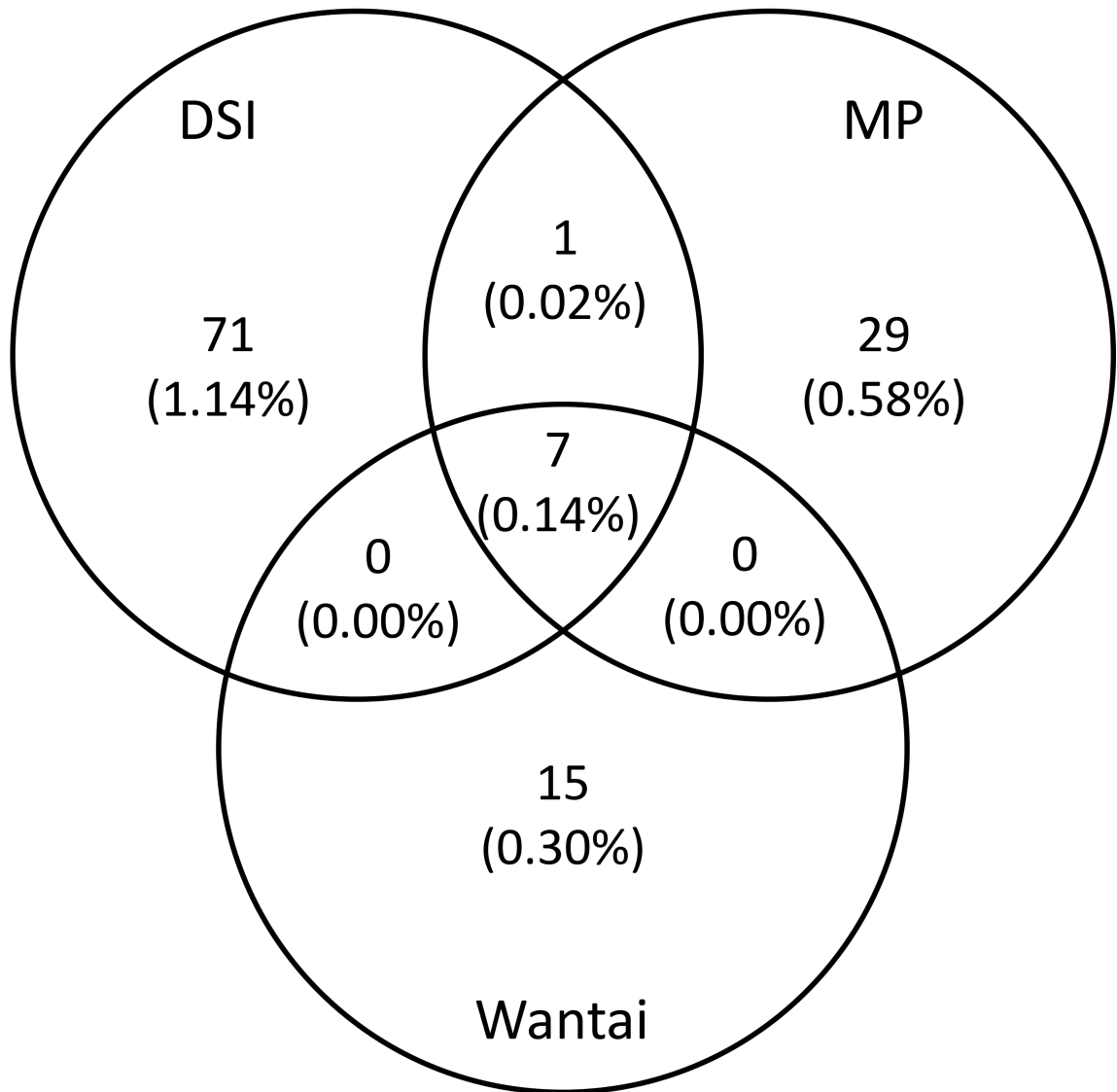Wantai
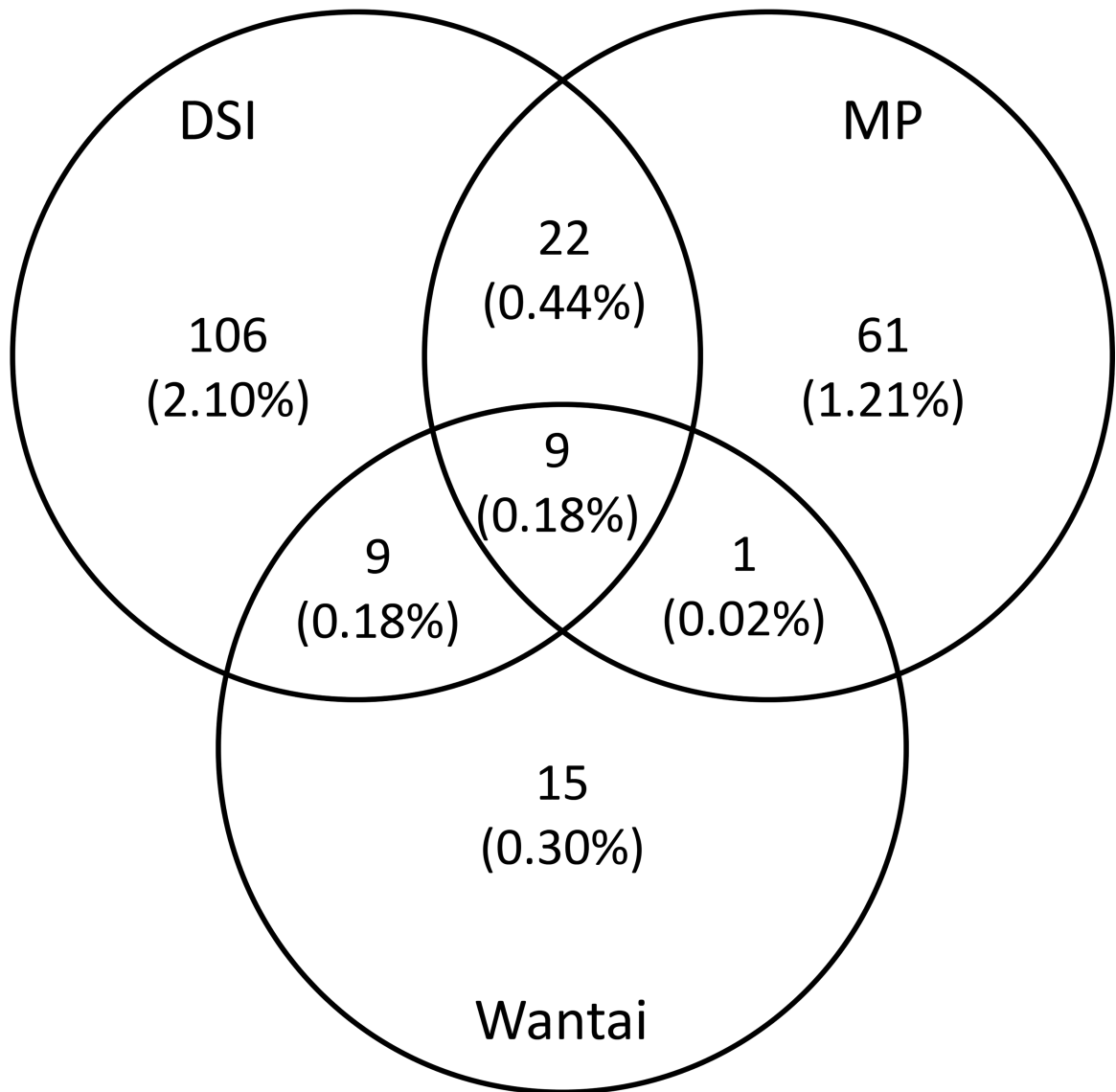
C. IgG/IgM

Figure 4.
Venn diagrams of anti-HEV assay concordance and discordance. A. IgG/total antibody assays, B. IgM assays and C. IgG/total antibody and IgM assays combined.
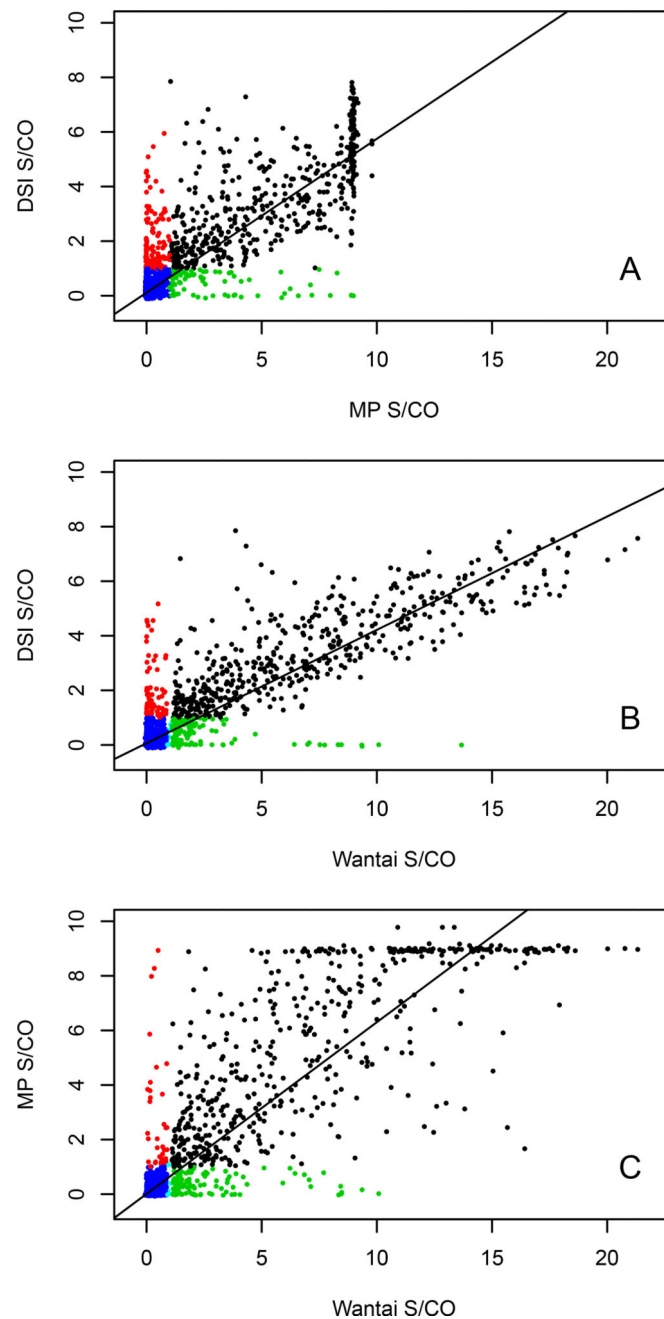
Figure 5.
Pairwise comparison of signal to cutoff (S/CO) specimen values among the DSI, MP
Biomedicals and Wantai IgG/total antibody assays. S/CO paired data are plotted as closed
circles. The solid line is the regression line through the data between each pair of assays.
Blue circles denote S/CO data pairs that are both non-reactive, black circles denote that both
results are reactive. A. Results from DSI compared with MP Biomedicals (red circles; DSI
reactive and MP non-reactive, green circles (DSI non-reactive and MP reactive)), B. DSI
compared with Wantai (red circles; DSI reactive and Wantai non-reactive, green circles (DSI
non-reactive and Wantai reactive)) and C. MP Biomedicals compared with Wantai (red

circles; MP reactive and Wantai non-reactive, green circles (MP non-reactive and Wantai reactive)).

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Table 1

Anti-HEV results by assay for 5040 blood donor samples. Results are listed as numbers per category followed by the percentage.

	Negative		Gray zone		Positive	
	IgG	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**
DSI	4471 (88.71%)	4894 (97.10%)	0 (0.00%)	0 (0.00%)	569 (11.29%)	146 (2.90%)[†‡]
MP	4503 (89.35%)[*]	4947 (98.15%)	0 (0.00%)[*]	0 (0.00%)	537 (10.65%)[*†]	93 (1.85%)[†‡]
Wantai	4415 (87.60%)	5006 (99.33%)	6 (0.1%)	0 (0.00%)	619 (12.28%)[†]	34 (0.67%)[†‡]
mean	88.55%	98.19%	0.04%	0.00%	11.41%	1.81%

[*] Assay detects total antibody rather than IgG alone.

[†] The MP Biomedicals (MP) and Wantai IgG assays show a significant difference at p < 0.025,

[‡] and all three IgM assays show significant differences between each other at p < 0.00075.

Table 2

Percent IgG antibody reactive donors by state and gender. The five states with the highest number of donors were compared.

State	Total*			Male			Female		
	DSI	MP	Wantai	DSI	MP	Wantai	DSI	MP	Wantai
Illinois	12.06	11.84	14.21	13.51	12.89	16.42	10.34	10.59	11.58
Indiana	9.47	8.36	10.21	11.14	9.55	11.82	7.51	6.97	8.31
Kansas	5.15	5.15	5.15	6.67	6.67	6.67	3.85	3.85	3.85
Kentucky	7.77	7.50	8.04	8.32	8.16	9.14	7.10	6.71	6.71
Missouri	13.89	13.13	15.00	15.74‡	13.89	16.20	11.90‡	12.30	13.70
All states†	11.29	10.65	12.28	12.68∥	11.48	13.65§	9.71∥	9.71	10.73§

*
Missouri (MO; n=2080), Kentucky (KY; n=1120), Illinois (IL; n=887), Indiana (IN; n=813) and Kansas (KS; n=97).

†
Seropositivity for all 21 states in which donors resided.

‡
$p < 0.05$;

§
$p < 0.01$;

∥
$p < 0.005$ (Chi-square for seropositivity by gender and assay).

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Table 3

Characteristics of the nine IgM anti-HEV positive specimens concordant across all three vendors.

sample	IgG S/CO			IgM S/CO			Age	Gender	State
	DSI	MP	Wantai	DSI	MP	Wantai			
1	0.01	0.08	0.13	3.03	4.61	1.42	18	M	KY
2	0.83	8.27	0.34	6.28	3.23	5.70	74	M	MO
3	6.28	8.99	14.14	1.73	1.13	2.31	68	M	MO
4	5.45	6.40	10.00	2.88	1.12	1.13	59	F	MO
5	5.18	9.00	16.60	3.33	1.44	1.12	47	M	KY
6	6.40	8.98	17.27	4.27	1.99	1.38	68	M	MO
7	5.51	8.44	14.34	4.66	2.31	7.30	81	F	MO
8	5.12	8.91	16.42	5.41	2.44	1.27	57	F	KY
9	2.29	8.91	6.33	7.84	5.09	2.65	63	M	MO

S/CO, signal/cutoff; age in years; State, KY – Kentucky, MO – Missouri. Samples 3 to 9 are concordant for IgG and IgM across all three vendors.

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