JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Al-Absi *et al.*, *Journal of Medical Microbiology* 2018;67:1302–1309

DOI 10.1099/jmm.0.000807

MICROBIOLOGY SOCIETY

Performance evaluation of five commercial assays in assessing seroprevalence of HEV antibodies among blood donors

Enas S. Al-Absi,[1,2] Duaa W. Al- Sadeq,[1] Manaf H. Younis,[3] Hadi M. Yassine,[2] Omnya M. Abdalla,[1] Areej G. Mesleh,[1] Tameem A. Hadwan,[1] Joshua O. Amimo,[4,5] Lukman Thalib[5] and Gheyath K. Nasrallah[1,2,*]

Abstract

Introduction. Although hepatitis E virus (HEV) is mainly transmitted via the faecal–oral route, the rate of HEV transmission via blood donation is on the rise. However, the seroprevalence of HEV among blood donors is not well established and is thought to be affected by the type of diagnostic assay used. We aimed to evaluate performance and correlation among widely used commercial diagnostic assays for the seroprevalence assessment of HEV-IgM/IgG among blood donors.

Methodology. A total of 1049 blood donor samples were tested for HEV IgG and IgM using different enzyme immunoassays (Wantai, Eruoimmune, MP diagnostics, Mikrogen immunoblot, HEV-IgM rapid test). The performance of each assay was evaluated according to our established silver standard value based on three or more IgG concordant assay results.

Results. HEV seroprevalence varied considerably using these assays, ranging from 10.1 % (Euroimmune-ELISA) to 18.0 % (Wanti-ELISA) for HEV-IgG, and from 0.2 % (Wanti-ELISA) to 2.6 % (MP Rapid test) for HEV-IgM. A total of 155 of 216 (71.6%) samples tested positive for HEV-IgG by three or more concordant assays. On the other hand, IgM assays showed poor agreement as only 7.6 % (4/52) of the specimens were positive according to three or more concordant assay test results. All HEV-IgG assays revealed high sensitivity and specificity (ranging 96.5–100 %),and excellent Kappa concordance (0.88–0.95), except for Euroimmun ELISA (sensitivity=61.5 %, kappa=0.63). MP ELISA showed the highest levels of sensitivity (100 %) and specificity (98.5 %).

Conclusions. Due to discrepancies in the performance of various IgG and IgM assays, seroprevalence studies should be based on furher confirmatory testing for decisive conclusions to be reached.

INTRODUCTION

HEV infection is among the most common causes of acute viral hepatitis worldwide. HEV is a small, non-enveloped, single-stranded, positive-sense RNA virus and known as enterically transmitted non-A, non-B hepatitis [1]. HEV belongs to the family Hepeviridae and genus *Orthohepevirus*. This genus is divided into four species containing multiple genotypes, where all the HEVs infecting humans belong to the species *Orthohepevirus* A. To date, four HEV genotypes from this species have been identified as infecting humans, and these are classified based on whole-genome analysis. Interestingly, all these four belong to one serotype according to neutralization assay [2–4]. In most cases, HEV infection is self-limiting and asymptomatic. However, in other cases, infection results in typical symptoms of acute hepatitis such as jaundice, hepatosplenomegaly and elevated liver transaminases [5]. According to the World Health Organization (WHO), 20 million new HEV cases are recorded annually. HEV-specific mortality is about 3.3 % worldwide, accounting for almost 44 000 deaths per year [1]. In addition, 10–30 % of pregnant women and more than 75 % of HEV-infected patients with underlying liver conditions die due to HEV infection [6–8].

HEV is mainly transmitted via the faecal–oral route, as well as by zoonotic transmission from infected animals including camel, deer, the domestic pig and wild boar [1].

Received 29 March 2018; Accepted 10 July 2018

Author affiliations: [1]Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar; [2]Biomedical Research Center, Qatar University, Doha, Qatar; [3]Department of Public Health, College of Health Sciences, Qatar University, Doha, Qatar; [4]Department of Animal Production, Faculty of Veterinary Medicine University of Nairobi, Nairobi, Kenya; [5]Bioscieces of Eastern and Central Africa-International Livestock Research Institute (BecA-ILRI), Nairobi, Kenya.

***Correspondence:** Gheyath K. Nasrallah, gheyath.nasrallah@qu.edu.qa

Keywords: HEV; IgG; IgM; Seroprevalence; Blood donors; EIA; Assay performance.

Abbreviations: AUC, Area Under Curve; ELISA, Enzyme-linked Immunosorbent Assay; HEV, Hepatitis E Virus; HMC, Hamad Medical Corporation; IgM, Immunoglobulin M; IgG, Immunoglobulin G; IRB, Institutional Review Boards; MENA, Middle East and North Africa; PCR, Polymerase Chain Reaction; QU, Qatar University; RNA, RiboNucleic Acid; ROC, Receiver Operating Characteristic; WHO, World Health Organization.

Interestingly, occasional transmission of HEV infection via blood transfusion has been documented as increasing in several countries around the world [9–15]. Therefore, blood transfusion is considered a potential risk for HEV spread, which poses a considerable threat to patients requiring blood transfusion [16]. Accordingly, HEV could represent a life-threatening infection in immune-compromised blood or organ recipients, and therefore there is a paucity of evidence for classifying HEV as an emergent blood pathogen regarding global blood supplies. Although detection of HEV antibodies is useful in the diagnosis of current or past HEV infection, studies have shown that these tests do not provide sufficient confirmatory evidence as to whether donor blood products are infectious and contain HEV-RNA [17, 18].

Although serological assays are more feasible in terms of cost and simplicity, their diagnostic sensitivity and specificity in detecting HEV are relatively low [19]. Accordingly, HEV-RNA detection remains the gold standard to determine viraemia [20, 21]. Clinically, RNA and antibody detection in the blood reflect different stages of a disease. HEV-RNA testing cannot be considered as a gold standard test to confirm assays that rely on antibody detection, such as IgG and IgM, because viraemia does not always coincide with the antibody response in the natural course of HEV infection. Recent studies suggest that conducting more scientific investigations, such as seroprevalence studies, is crucial to verifying whether additional methods for HEV-RNA detection in blood banks are necessary [18]. Moreover, using an expensive test such as HEV-RNA PCR on a large population of blood donors, or during outbreaks, as a routine screening tool is economically inefficient. Therefore, in this study there was a pressing need to establish a silver standard as an equivalence reference or confirmatory assay to evaluate the performance of antibody assays [22]. In this approach, the sample is considered positive only if testing positive by three or more different immunoassays.

There is currently no FDA-approved serological assay for HEV clinical diagnosis. The available commercial assays can only aid in screening for HEV and can be used in epidemiological studies, yet none is known to be confirmatory [4]. More critically, previous studies have observed that seroprevalence among healthy or acute hepatitis individuals is markedly affected by the type of laboratory assay [4, 16]. This led to a huge variation in seroprevalence in the same population. For instance, it was noted in three different studies conducted on the same study population over the same period in Germany that the seroprevalence of HEV IgG ranged between 6 and 16 % [23–25]. Likewise, studies in which commercial assays were used for detection of HEV IgM, among acute hepatitis samples, resulted in wide variation in diagnostic performance [26–28]. Accordingly, HEV seroprevalence is affected not only by the study population, geographic distribution and the time period (season), but also by the type of detection assay used. Therefore, this study aimed to assess the concordance between widely used commercial assays that rely on the detection of IgM/IgG to assess HEV seroprevalence among large populations of blood donors from different nationalities and backgrounds, and to evaluate their performance as a screening tool in the absence of the costly gold standard PCR.

METHODS

Ethical compliance and sample collection

Ethical clearance was obtained from the Hamad Medical Corporation (HMC) and Qatar University (QU) Institutional Review Boards (HMC-IRB #14292/14 and QU-IRB #556-EA/16, respectively) prior to the study sample collection. A total of 1049 blood samples of apparently healthy blood donors was obtained from the blood donation centre of the HMC during the period June 2013–June 2016. The blood samples were originally collected for other studies [16, 29–32]. The sampling, transport and storage methods and details of the study population were described previously [16, 29, 31–33].

Assays used for detection of HEV antibodies

Enzyme-linked immunosorbent assay (ELISA)

Commercial ELISA kits from four different companies were used for qualitative detection of HEV IgG and IgM antibodies in the sera of blood donors:

(1) **MP Dignostics** (HEV-IgG) ELISA catalogue no. 0721150096T, HEV-IgM ELISA 3.0 catalogue no. 0723160096, and HEV-Total antibody ELISA 4.0 catalogue no.0723540096T, California, USA)
(2) **Wantai** (HEV-IgM) ELISA catalogue no. WE-7196 and HEV IgG ELISA catalogue no. WE-7296, Beijing, China)
(3) **Euroimmun** (HEV-IgG) ELISA catalogue no. EI 2525 G, and HEV IgM ELISA catalogue no. EI 2525 M, Lübeck, Germany).

HEV Immunoblot assay

RecomLine HEV by Mikrogen (HEV IgG\IgM recomLine catalogue no. 5070, Bavaria Germany) is an *in vitro* qualitative strip immunoassay coated with recombinant antigens. The test is used to detect HEV-IgG or IgM antibodies in human serum or plasma. Analysis was performed according to the manufacturer's instructions.

MP diagnostics assure IgM rapid assay

MP Biomedicals (catalogue no. 0743160020, CA, USA). MP Diagnostics ASSURE IgM rapid test is an immune-chromatographic test that offers rapid detection of IgM-HEV antibodies in human serum, plasma or whole blood. Analysis was performed according to the manufacturer's instructions.

Table 1 provides a comparison between the assay kits used in this study in terms of coating of the wells, detection of antibodies and the starting dilution, time of incubation and cut-off value. Testing using the above-mentioned kits was performed according to each manufacturer's instructions and protocols. A microplate reader, Epoch 2 microplate

Table 1. Comparison among different immunoassays

	Coating of wells	Detection	Starting dilution	Incubation time	Cut-off
MP IgG/IgM	**IgG kit:** three recombinant HEV antigens*† **IgM kit:** highly conserved conformational epitope derived from ORF3†	**IgG:** labelled goat anti-human IgG **IgM:** labeled monoclonal mouse anti-human IgM	1 : 20 10 µl of the sample in 200 µl of dilution buffer	Samples incubated into micro-wells: 30 min. Incubation with conjugate: 30 min at 37 °C	Calculated as: 0.5 absorbance unit (for IgG) and 0.4 (for IgM)+mean absorbance of negative controls
Euroimmun IgG/IgM	Recombinant antigens from HEV genotype 1 and 3*	**IgG**: labelled goat anti-human IgG **IgM**: labelled rabbit anti-human IgM	1 : 100 100 µl of the sample	Samples incubated into micro-wells: 30 min. Incubation with conjugate: 30 min at RT	A calibrator material was used as a cut-off
Wantai IgG/ IgM	IgG: recombinant HEV antigens*† IgM: human immunoglobulin M proteins (anti-µ chain)	**IgG**: labelled anti-human IgG **IgM**: labelled recombinant HEV ORF2 antigen	1 : 10 10 µl of the sample	Samples incubated into micro-wells: 30 min. Incubation with conjugate: 30 min at 37 °C	Calculated as: mean absorbance of negative controls+0.16 or (0.26 for IgM)
MP Rapid test	Immobilized colloidal gold-labelled recombinant HEV antigen*†	HEV **IgM** antibodies	35 µl of the sample added then one drop of chase buffer	Samples incubated in cassette for 15 min at RT	–
Mikrogen immunoblot	ORF2 N and C- terminal part antigens from genotype 1 and 3; ORF2 middle part antigen from genotype1; and ORF3 antigens from genotype 1 and3	**IgG:** labelled anti-human IgG **IgM:** labelled anti-human IgM	1:100 20 µl of the sample in 2 ml of dilution buffer	Samples incubated with test strep contains 7 antigen bands: 1 h. Incubation with conjugate: 45 min at 37°C	Colour intensity of the bands for the test compared to positive control

*Antigen derived from unknown ORF.
†Antigen derived from unknown genotype(s).

spectrophotometer (Bio-Tek, Italy), was used to read the optical density in all ELISA reactions. Borderline (equivocal) samples were repeated in duplicate. Subsequently, if one or both repeated tests were positive, the sample was considered positive. However, if the two repeated tests were negative, the sample was considered negative. In cases where both repeated tests revealed borderline values, the sample was considered negative.

Relative performance (titre quantification)

Relative performance (titre quantification) was performed to determine the least amount of anti-HEV concentration (titre detection limit) detectably by each assay. Five samples testing positive for HEV-IgG by all serological assays (Table 5) were chosen randomly regardless of their IgM test results. Tests were performed on 10-fold serially diluted (1 : 10, 1 : 100, 1 : 1000 and 1 : 10 000) samples [28, 34]. A 1 : 5000 dilution was performed on samples that were negative at 1 : 10 000 dilution. Finally, a score of 1 to 5 was given to any positive result based on the sensitivity in detecting the lowest anti-HEV concentration (titrate). Thus, a score of 5 represents an anti-HEV concentration that was detected at the highest dilution (lowest titrate).

Diagnostic performance evaluation

IgG antibodies were tested since HEV-IgG persists for a long period following exposure/clearance of HEV infection. Due to the absence of approved HEV-IgG confirmatory tests, we developed a 'silver standard' evaluation to be used for diagnosis of HEV, after referring to senior expert opinion in the field. The main idea behind the silver standard was to increase the probability of true positives (TPs) and true negatives (TNs), and to decrease the probability of false positives (FPs) and false negatives (FNs). In the proposed silver standard test, only samples that tested positive by three or more different sets of assay were considered positive. Diagnostic performance evaluation for the HEV IgG assays was then determined with respect to the silver standard that we had developed. Diagnostic sensitivity, specificity and efficiency were calculated for each assay against the silver standard as follows: sensitivity = [true positive/(true positive + false negative)*100]; specificity = [true negative/(true negative + false positive)*100]; efficiency = [(true positive + true negative)/(true positive + true negative + false positive + false negative)] for each serological assay used to detect HEV IgM.

Statistical analysis

Cohen's kappa value was calculated to determine the level of agreement of the assays used for blood donors. In addition, ROC curve analysis was used to obtain the area under the curve (AUC) for the assays to determine the ideal cut-off value that would enhance diagnostic accuracy. AUC expresses the accuracy of the test in discriminating positive (diseased) and negative values (none diseased). Statistical Package for the Social Sciences (SPSS) software version 23.0 was used to analyse the quantitative data.

RESULTS

A total of 1049 samples from blood donors of different nationalities (*n*=83) was included in this study. The demographic characteristics of the blood donors are summarized in Table 2. The seroprevalence and current disease rate varied by age and nationality. This was supported by another study where HEV seroprevalence increased with age and was more prevalent in Sudanese and Pakistani patients [16]. Initially, seroprevalence was determined for both IgG and IgM antibodies using three different ELISA assays (Wantai, MP and Euroimmun) and one immunoblot-based assay (Mikrogen). Table 3 shows the differences in seroprevalence for IgG and Igamong the four serological assays, in which IgG and IgM seroprevalence ranged from 10.1–18.0 % and 0.2–2.4 %, respectively. We observed marked variation in the seroprevalence values obtained using different HEV-IgG assays (Table 3). Wantai had the highest detection rate (18.0 %), followed by Mikrogen (17.7 %), MP ELISA (16.1 %) and Euroimmun (10.1 %). The variation was more obvious among the HEV-IgM-based assays. The MP rapid test had the highest detection rate for HEV-IgM (2.6 %), followed by Mikrogen (2.4 %), MP (2.1 %), Euroimmun (0.4 %) and Wantai (0.2 %). Furthermore, there was a 13-fold difference in the HEV-IgM detection rate between the MP rapid test (*n*=27) and Wantai (27 vs 2, respectively).

Subsequently, the diagnostic sensitivity, specificity and efficiency of each kit were calculated against the silver standard to determine the performance characteristics of HEV-IgG assays. As shown in Table 4, all assays showed sensitivity values above 98 %, except for Euroimmun, which had a sensitivity value of 61.5 %. However, the four assays showed high specificity values, above 96 % (Table 4). The assays were ranked according to their diagnostic efficiency in the following order: MP ELISA (98.7 %), Wantai ELISA (97.0 %), Mikrogen immunoblot (96.7 %) and Euroimmun ELISA (93.3 %). Furthermore, the agreement between the assays and the silver standard (confirmatory) test for detection of HEV IgG (Table 4) was acceptable. The kappa values were 0.95 for MP ELISA, 0.89 for Wantai ELISA, 0.88 for Mikrogen immunoblot and 0.69 for Euroimmun ELISA, while 71.6 % (155/216) of samples tested positive for HEV-IgG by three or more concordant assays (Table 5). On the other hand, IgM assays showed poor agreement as only 7.6 % (4/52) of the specimens were positive by three or more concordant assays test results.

Table 2. Demographic characteristics of randomly selected blood donor samples (*n*=1049)

Characteristic		No. (%)
Gender	Female	77 (7.3)
	Male	672 (92.7)
Age	15–24	119 (11.3)
	25–34	406 (38.7)
	35–44	354 (33.7)
	45–54	134 (12.8)
	55+	36 (3.4)
Nationality	Qatari	249 (23.7)
	Indian	188 (17.9)
	Egyptian	125 (11.9)
	Asian	117 (11.1)
	Western	33 (3.1)
	Others	337 (32.1)

The Asian group includes subjects from Sirlanka, Bangladesh, Nepal, Philippines and Pakistan.

The Western group includes subjects from the USA, the UK, Brazil, France, Germany, Ireland, Italy, the Netherlands, Spain, Romania, New Zealand and Australia.

Others: the majority from MENA not included above.

Relative performance (Titre quantitation) of different HEV-IgG diagnostic assays using anonymous positive donor samples

Relative performance was calculated for each assay in order to compare the HEV-IgG detection limits of all assays. The relative performance, from five randomly selected HEV-IgG-positive samples, was determined (Table 6). The samples were selected based on testing positive by all four HEV-IgG assays. The highest detection limit (titre) for HEV-IgG was identified by Mikrogen, at a dilution of 1 : 10 000 (titre score of 19), followed by Euroimmun at a dilution of 1 : 1000 (titre score of 11).

DISCUSSION

We analysed HEV seroprevalence using different immunoassays to develop a better evaluation tool for the diagnosis and estimation of the seroprevalence of HEV infection. Moreover, this study includes data on subjects of different nationalities, as the actual Qatari population accounts for less than 20% of the study population (Table 2). A major element of this study is the inclusion of a large sample size of blood donors representing countries from around the globe, to evaluate the seroprevalence of anti-HEV IgM and IgG using a range of immunological binding assays. The seroprevalence of HEV ranged 0.2–2.6% and 10.3–18.1 % for IgM and IgG, respectively. This marked discrepancy between assays has a substantial impact on estimation of the actual seroprevalence of HEV in any population.

As none of the assays used is considered a confirmatory test *per se*, we propose a silver standardization protocol, incorporating consultation with experts in virology, that is based on the results of multiple tests. This approach provides a better assessment among assays when a confirmatory test is lacking. A silver standard was used to evaluate the performance of different serological assays. It gives a baseline and guidance for other laboratories to select the highest-performing assay. However, PCR is usually used to measure the level of viraemia but HEV RNA in samples does not always correspond to antibody levels. Moreover, the high cost of running PCR as a screening tool on a large population of blood donors puts a huge financial burden on health system resources. Consequently, a performance

Table 3. Seroprevalence of HEV IgG and HEV IgM according to different serological assays among blood donors (*n*=1049)

Assay	HEV IgM (n)	% (95% CI)	HEV IgG (n)	% (95% CI)
MP ELISA	22	2.1 (1.2–2.9)	169	16.1 (14–18.4)
Mikrogen immunoblot	25	2.4 (1.5–3.3)	186	17.7 (15.5–20.1)
Wantai ELISA	2	0.2 (0.1–0.4)	189	18.1 (15.7–20.3)
Euroimmun ELISA	4	0.4 (0.01–0.7)	108	10.3 (8.5–12.2)
MP HEV-IgM Rapid test	27	2.6 (1.6–3.5)	–	–
Silver standard*	–	–	**156**	14.9 (12.7–17)

n: number of samples.

*Silver standard: confirmatory test in which samples positive (*n*=156) or negative (*n*=889) by at least three assays were considered true positive (TP) or true negative (TN), respectively. Silver standard was not performed for IgM, since the number of positive samples was very low and thus the silver standard could not be established.

a, MP Total: measures HEV IgG, IgM and IgA.

b, Wantai Ag: detects HEV antigen.

characteristic for each assay was calculated with respect to the silver standard. As illustrated in Table 4, MP IgG-ELISA showed the highest sensitivity, specificity, overall agreement with the silver standard and kappa value, followed by Wantai ELISA. A seroprevalence score of 16.1 % was yielded by MP-IgG ELISA, which is the closest score yielded by the silver standard (14.9 %) (Table 3); interestingly the MP IgG kit is the only one in which the wells are coated with three different recombinant proteins (Table 1). Overall, all the other kits have high sensitivity, specificity and a narrow confidence interval when compared to the silver standard. However, Euroimmun ELISA possessed the lowest sensitivity, which may be due to its cut-off determination criteria or even the antigen concentration of the coated wells. This could be due to the starting dilution, which was 1 : 100. The finding may rule out the concentration issue, but rather, questions the efficiency of coated peptides in detecting different antibody responses by diverse HEV genotypes. Additionally, Euroimmun showed poor agreement with the silver standard.

The seroprevalnce of HEV IgM was examined using four different serological assays. A total of 52 samples were HEV IgM-positive out of 1049 samples (Table 7). Similarly, a previous seroprevalence study examining 5854 blood donor samples revealed that only 32 tested positive for HEV IgM. However, only four samples were confirmed by RT-PCR [16]. This suggests that detection of HEV IgM in blood donors with acute infection may not be accurate since RNA and IgM antibody titres vary between different HEV infection stages. In addition, the donor status could be either (i) virus free/antibody free, (ii) virus free/antibody positive (recovered and cleared infection), (iii) virus positive/antibody negative (acute infection, too early for antibody production or detection) or (iv) virus positive/antibody positive (recovering). Therefore, these assays cannot accurately determine iwhether active infection is present, nor can they accurately detect acute

Table 4. Diagnostic performance of four HEV IgG serological assays among blood donors (*n*=1049)

	Sensitivity (95% CI)	Specificity (95% CI)	Efficiency (%)*	Kappa value†	Overall agreement (%)	Standard error of kappa value	ROC curve analysis (AUC)‡
MP ELISA	100.0 (97.7–100.0)	98.5 (97.5–99.2)	98.7	**0.95**	**98.8**	**0.01**	**0.98**
Wantai ELISA	100.0 (97.7–100.0)	96.5 (95.1–97.6)	97.0	0.89	97.0	0.02	0.98
Mikrogen immunoblot	98.1 (94.5–99.6)	96.5 (95.1–97.6)	96.7	0.88	96.7	0.02	0.97
Euroimmun ELISA	**61.5** (53.4–69.2)	98.8 (97.8–99.4)	93.3	**0.69**	**93.2**	**0.03**	**0.95**

Note: sensitivity, specificity and efficiency were calculated against the silver standard. CI, confidence interval.

*Efficiency: [(TP+TN)/(TP+TN+FP+FN)]

†Cohen's Kappa value: a measure of agreement level between each assay and the silver standard. The higher the value (or the closest to 1.0), the stronger the agreement.

‡AUC: area under curve obtained from ROC curve analysis to determine the accuracy of the assay; more related to positive and negative predictive values than sensitivity.

Table 5. Proportions of positive samples for HEV IgM and IgG using different immunoassays

Total positive samples (%)		Positive by three or more assays (%)		Positive by two assays (%)		Positive by one assay (%)	
IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
216 (20.6)	52 (5)	155/216 (71.8)	4/52 (7.6)	30/216 (13.9)	17/52 (32.6)	31/216 (14.3)	31/52 (59.6)

infection prior to antibody production; or the threshold levels of the assays used may be exceeded. The use of the imperfect gold standard 'silver standard' could lead to what is called 'imperfect gold standard bias'. The effect of this bias depends on whether the errors on the 'silver standard' are correlated with errors on the index test, which might expose the results to misclassifications leading to falsely decreased sensitivity and specificity [35].

The above-mentioned results are not in line with the relative performance analysis, since Mikrogen and Euroimmun were able to detect IgG in samples diluted up to 1 : 10 000 and 1 : 1000, respectively. On the other hand, the MP and Wantai assays were only able to detect IgG in samples diluted up to 1 : 10 and 1 : 100, respectively. This is dependent on many factors, including: the antigen type and the amount coated on the plate; the nature of secondary antibodyies; whether a monoclonal or polyclonal antibody was used; nd the incubation period. Although there is only one serotype, commercial assays are designed to detect different recombinant epitopes (ORF1, ORF2, ORF3) or combinations thereof that are derived from different genotypes (genotypes 1 to 3). Although genotype 3 is the most prevalent genotype worldwide, no data areon the most prevalent genotype in the Gulf region. This could lead to discrepancies between assays and lack of sensitivity, particularly in our study where we used samples from different nationalities, mainly from Asia and the MENA region. As noted in Table 1, both Mikrogen and Euroimmun recommend diluting the sample 100-fold. Although Wantai and MP ELISAs use 10- and 20-fold dilution, respectively, their detection limits were lower than Mikrogen and Euroimmun. Even though the assays that recommend using a lower sample to diluent ratio require a longer incubation period, this did not improve the sensitivity issue. All assays recommend incubation with the conjugate for 30 min. In addition, MP ELISA showed the highest sensitivity and specificity among other immunoassays. This could be due to the fact that this assay utilizes antigens encoded by ORF3. These antigens represent the surface (glycoproteins) of the virus, which are the primary target of the antibody response, and this partly suggests the adequacy of using these in binding assays [36]. In contrast, other kits utilize the ORF2 antigen, which is the virus capsid protein.

In conclusion, the available serological assays of HEV antibodies show discrepancies in their results. Therefore, the precise seroprevalence of HEV antibodies remains to be determined. In addition, these assays cannot be used individually to provide an accurate diagnosis of HEV infection, due to the wide variation in their sensitivities and specificities. Despite the fact that RT-PCR is considered the gold standard for the diagnosis of acute HEV infections and for determining the level of viraemia in blood donors, it has several limitations including high cost and the requirement of expertise, especially in the context of developing countries where resources are scarce. Owing to funding limitations, it was not practical in this study to perform RT-PCR for the 1049 samples. It should be clarified that serology assays detect anti-HEV antibodies using various antigens at different stages of active or prior HEV infection. However, HEV infection could be passed on during blood product transfusion when antibody levels are 'negative' but the pathogen is present – i.e. viraemia. Therefore, we recommend selective screening of blood donors for HEV RNA, especially for high-risk recipients such as immunocompromised and pregnant women, to prevent virus-positive donors from providing products for blood transfusion. Furthermore, standardization of the diagnostic procedures and the availability of efficiency panels are required to achieve accurate assessment of HEV seroprevalence and diagnosis.

Table 6. Relative performance (detection limit) of different serological assays (*n*=5)

Sample no.	Wantai IgG	MP Diagnostic	Mikrogen	Euroimmun
190 p1	1	1	3	2
205 p1	1	1	3	2
243 p1	2	1	5	3
330 p1	2	1	3	3
402 p1	2	1	5	2
Total titre score	8	5	**19**	11

Note: **1**: <1:10, **2**: 1:100, **3**: 1:1,000, **4**: 1:5,000, **5**: 1: 10,000.

Table 7. HEV-IgM samples testing positive by each assay (*n*=1049)

Sample	Wantai IgM	Euroimmun IgM	Mikrogen blot	MP IgM	MP Rapid IgM	Silver Standard
230 P1	–	+	+	+	+	+
885 P1	–	–	+	+	+	+
899 P1	+	–	+	+	+	+
1046 P1	–	+	+	+	+	+
210 P1	–	–	+	+	–	–
222 P1	–	–	+	+	–	–
324 P1	–	–	+	+	–	–
984 P1	–	–	+	+	–	–
711 P1	–	–	+	+	–	–
228 P1	–	–	+	–	+	–
239 P1	–	–	+	–	+	–
399 P1	–	–	+	–	+	–
404 P1	–	–	+	–	+	–
405 P1	–	–	+	–	+	–
282 P1	–	–	+	–	+	–
355 P1	–	–	+	–	+	–
471 P1	–	–	+	–	+	–
479 P1	–	–	+	–	+	–
851 P1	–	–	+	–	+	–
759 P1	–	–	+	–	+	–
953 P1	–	–	–	+	+	–
496 P1	+	–	–	–	–	–
255 P1	–	+	–	–	–	–
998 P1	–	+	–	–	–	–
369 P1	–	–	+	–	–	–
370 P1	–	–	+	–	–	–
760 P1	–	–	+	–	–	–
893 P1	–	–	+	–	–	–
105 P1	–	–	+	–	–	–
149 P1	–	–	–	+	–	–
468 P1	–	–	–	+	–	–
507 P1	–	–	–	+	–	–
556 P1	–	–	–	+	–	–
654 P1	–	–	–	+	–	–
750 P1	–	–	–	+	–	–
838 P1	–	–	–	+	–	–
960 P1	–	–	–	+	–	–
967 P1	–	–	–	+	–	–
982 P1	–	–	–	+	–	–
1036 P1	–	–	–	+	–	–
1037 P1	–	–	–	+	–	–
88 P1	–	–	–	–	+	–
42 P1	–	–	–	–	+	–
164 P1	–	–	–	–	+	–
175 P1	–	–	–	–	+	–
243 P1	–	–	–	–	+	–
303 P1	–	–	–	–	+	–
374 P1	–	–	–	–	+	–
449 P1	–	–	–	–	+	–
694 P1	–	–	–	–	+	–
736 P1	–	–	–	–	+	–
1032 P1	–	–	–	–	+	–

Funding information
The Authors would like to thank Ms. Nadiam Haj Ali for her technical assistance. This study was supported by UREP grant given to GKN. This report was made possible by UREP grant # UREP19-013-3-001 from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of the author(s).

Ethical statement
The work presented in this manuscript involved work with human blood samples. Ethical approval was obtained from Institutional Review Board office in Qatar University.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References
1. WHO. 2016. Hepatitis E. www.who.int/mediacentre/factsheets/fs280/en/ [accessed 2016]
2. Emerson SU, Clemente-Casares P, Moiduddin N, Arankalle VA, Torian U *et al.* Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J Gen Virol* 2006;87:697–704.
3. Engle RE, Yu C, Emerson SU, Meng XJ, Purcell RH. Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol* 2002;40:4576–4580.
4. Al-Sadeq DW, Majdalawieh AF, Nasrallah GK. Seroprevalence and incidence of hepatitis E virus among blood donors: A review. *Rev Med Virol* 2017:e1937.
5. Aggarwal R. Diagnosis of hepatitis E. *Nat Rev Gastroenterol Hepatol* 2013;10:24–33.
6. Taherkhani R, Farshadpour F. Epidemiology of hepatitis E in pregnant women and children in Iran: a general overview. *J Clin Transl Hepatol* 2016;4:269–276.
7. Marano G, Vaglio S, Pupella S, Facco G, Bianchi M *et al.* Hepatitis E: an old infection with new implications. *Blood Transfus* 2015;13:6–17.
8. Taherkhani R, Farshadpour F. A new strategy for development of hepatitis E vaccine: epitope-based vaccines. *Pathogen & Infectious Disease* 2015.
9. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D *et al.* Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfus Med* 2006;16:79–83.
10. Colson P, Coze C, Gallian P, Henry M, De Micco P *et al.* Transfusion-associated hepatitis E, France. *Emerg Infect Dis* 2007;13:648–649.
11. Khuroo MS, Kamili S, Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. *J Gastroenterol Hepatol* 2004;19:778–784.
12. Matsubayashi K, Kang JH, Sakata H, Takahashi K, Shindo M *et al.* A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. *Transfusion* 2008;48:1368–1375.
13. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K *et al.* Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. *Transfusion* 2004;44:934–940.
14. Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F *et al.* Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. *J Med Virol* 2004;74:563–572.
15. Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y *et al.* Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatol Res* 2007;37:113–120.
16. Nasrallah GK, Al Absi ES, Ghandour R, Ali NH, Taleb S *et al.* Seroprevalence of hepatitis E virus among blood donors in Qatar (2013-2016). *Transfusion* 2017;57:1801–1807.
17. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 2008;48:494–503.
18. Féray C, Pawlotsky JM, Roque-Afonso AM, Samuel D, Dhumeaux D. Should we screen blood products for hepatitis E virus RNA? *Lancet* 2014;383:218.
19. Al-Sadeq DW, Majdalawieh AF, Mesleh AG, Abdalla OM, Nasrallah GK. Laboratory challenges in the diagnosis of hepatitis E virus. *J Med Microbiol* 2018:466–480.
20. Vollmer T, Knabbe C, Dreier J. Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors. *J Clin Microbiol* 2014;52:2150–2156.
21. Gupta E, Pandey P, Pandey S, Sharma MK, Sarin SK. Role of hepatitis E virus antigen in confirming active viral replication in patients with acute viral hepatitis E infection. *J Clin Virol* 2013;58:374–377.
22. Kohn MA, Carpenter CR, Newman TB. Understanding the direction of bias in studies of diagnostic test accuracy. *Acad Emerg Med* 2013;20:1194–1206.
23. Pischke S, Behrendt P, Bock CT, Jilg W, Manns MP *et al.* Hepatitis E in Germany - an under-reported infectious disease. *Dtsch Arztebl Int* 2014;111:577–583.
24. Faber MS, Wenzel JJ, Jilg W, Thamm M, Höhle M *et al.* Hepatitis E virus seroprevalence among adults, Germany. *Emerg Infect Dis* 2012;18:1654–1657.
25. Wenzel JJ, Sichler M, Schemmerer M, Behrens G, Leitzmann MF *et al.* Decline in hepatitis E virus antibody prevalence in southeastern Germany, 1996-2011. *Hepatology* 2014;60:1180–1186.
26. Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF *et al.* Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. *J Clin Virol* 2013;58:629–634.
27. Park HK, Jeong SH, Kim JW, Woo BH, Lee DH *et al.* Seroprevalence of anti-hepatitis E virus (HEV) in a Korean population: comparison of two commercial anti-HEV assays. *BMC Infect Dis* 2012;12:142.
28. Avellon A, Morago L, Garcia-Galera del Carmen M, Munoz M, Echevarría JM. Comparative sensitivity of commercial tests for hepatitis E genotype 3 virus antibody detection. *J Med Virol* 2015;87:1934–1939.
29. Al-Qahtani AA, Alabsi ES, Abuodeh R, Thalib L, El Zowalaty ME *et al.* Prevalence of anelloviruses (TTV, TTMDV, and TTMV) in healthy blood donors and in patients infected with HBV or HCV in Qatar. *Virol J* 2016;13:208.
30. Amimo JO, El Zowalaty ME, Githae D, Wamalwa M, Djikeng A *et al.* Metagenomic analysis demonstrates the diversity of the fecal virome in asymptomatic pigs in East Africa. *Arch Virol* 2016;161:887–897.
31. Abuodeh RO, Al-Absi E, Ali NH, Khalili M, Al-Mawlawi N *et al.* Detection and phylogenetic analysis of human pegivirus (GBV-C) among blood donors and patients infected with hepatitis B virus (HBV) in Qatar. *J Med Virol* 2015;87:2074–2081.
32. Abuodeh R, Al-Mawlawi N, Al-Qahtani AA, Bohol MF, Al-Ahdal MN *et al.* Detection and genotyping of torque teno virus (TTV) in healthy blood donors and patients infected with HBV or HCV in Qatar. *J Med Virol* 2015;87:1184–1191.
33. Smatti MK, Yassine HM, Abuodeh R, Almarawani A, Taleb SA *et al.* Prevalence and molecular profiling of Epstein Barr virus (EBV) among healthy blood donors from different nationalities in Qatar. *PLoS One* 2017;12:e0189033.
34. Abravanel F, Chapuy-Regaud S, Lhomme S, Dubois M, Peron JM *et al.* Performance of two commercial assays for detecting hepatitis E virus RNA in acute or chronic infections. *J Clin Microbiol* 2013;51:1913–1916.
35. Staquet M, Rozencweig M, Lee YJ, Muggia FM. Methodology for the assessment of new dichotomous diagnostic tests. *J Chronic Dis* 1981;34:599–610.
36. Cao D, Meng XJ. Molecular biology and replication of hepatitis E virus. *Emerg Microbes Infect* 2012;1:e17.