Diagnostic performance of a fully automated hepatitis E virus antibody immunoassay

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Abstract

In immunocompetent patients with liver inflammation, the detection of IgG and IgM antibodies against the hepatitis E virus (HEV) is important for the diagnosis. Recently, fully automated chemiluminescence immunoassays (CLIAs, LIAISON® Murex anti-HEV IgG/anti-HEV IgM test, DiaSorin) have become available for this purpose. The diagnostic suitability of these CLIAs was determined by comparison with a combination of plate-based enzyme immunoassays (*recom*Well HEV IgG/IgM ELISA, Mikrogen) and immunoblots (*recom*Line HEV IgG/IgM, Mikrogen), which served as a reference for the characterization of sera. Samples with a deviating result were retested with an alternative test (WANTAI HEV IgG/IgM ELISAs). The anti-HEV IgG CLIA had a sensitivity and specificity of 100% (100/100; 49/49) each when the *recom*Well HEV IgG ELISA served as a reference. The anti-HEV IgM CLIA had a sensitivity of 67.9% (36/53) and a specificity of 100% (49/49). When IgM immunoblot results were considered, sensitivity and specificity were 88.9% (24/27) and 53.8% (14/26), respectively. The WANTAI test confirmed 52.9% (9/17) of negative CLIA IgMs that differed from the *recom*Well HEV IgM result. The CLIA revealed an isolated and thus probably non-specific HEV IgM in one of 17 patients with acute Epstein-Barr virus infection. The automated CLIAs are well suited for HEV diagnostics.

Title: Diagnostic performance of a fully automated hepatitis E virus antibody immunoassay

Running title: Performance of automated HEV antibody tests

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Abstract

In immunocompetent patients with liver inflammation, the detection of IgG and IgM antibodies against the hepatitis E virus (HEV) is important for the diagnosis. Recently, fully automated chemiluminescence immunoassays (CLIAs, LIAISON® Murex anti-HEV IgG/anti-HEV IgM test, DiaSorin) have become available for this purpose. The diagnostic suitability of these CLIAs was determined by comparison with a combination of plate-based enzyme immunoassays (*recom* Well HEV IgG/IgM ELISA, Mikrogen) and immunoblots (*recom* Line HEV IgG/IgM, Mikrogen), which served as a reference for the characterization of sera. Samples with a deviating result were retested with an alternative test (WANTAI HEV IgG/IgM ELISAs). The anti-HEV IgG CLIA had a sensitivity and specificity of 100% (100/100; 49/49) each when the *recom* Well HEV IgG ELISA served as a reference. The anti-HEV IgM CLIA had a sensitivity of 67.9% (36/53) and a specificity of 100% (49/49). When IgM immunoblot results were considered, sensitivity and specificity were 88.9% (24/27) and 53.8% (14/26), respectively. The WANTAI test confirmed 52.9% (9/17) of negative CLIA IgMs that differed from the*recom* Well HEV IgM result. The CLIA revealed an isolated and thus probably non-specific HEV IgM in one of 17 patients with acute Epstein-Barr virus infection. The automated CLIAs are well suited for HEV diagnostics.

Keywords

Hepatitis E virus, Immunoassays, Sensitivity, Specificity, Polyclonal Stimulation

Abbreviations

CMV – Cytomegalovirus

EBV – Epstein-Barr virus

GT – Genotype

HEV – Hepatitis E virus

Introduction

The hepatitis E virus (HEV) belongs to the species *Orthohepevirus*A within the Hepeviridae family and has a single-stranded positive-sense RNA genome. HEV is prevalent worldwide and is considered one of the main causes of viral acute hepatitis [1]. So far, eight HEV genotypes (gt) have been distinguished, which differ in their host tropism and epidemiology [1]. In Germany and some other countries in Europe and North America, gt 3 in particular is endemic. Domestic and wild pigs represent an important animal reservoir for this genotype [1,2]. The most important source of infection for humans is the consumption of raw or insufficiently cooked meat. Other transmission routes are direct animal contact, consumption of water or agricultural products contaminated with manure, organ transplants and blood transfusions [3]. Under immunosuppression, infections with gt 3 (and rarely gt 4) can become chronic [1]. In contrast, gt 1 and 2 are limited to humans as hosts and are rarely detected in industrialized countries. Infections with these types are considered travel-related, especially since major outbreaks have been reported in regions with poor hygienic conditions [1]. The number of HEV infections reported annually is steadily increasing in many industrialized countries, mainly due to increased awareness among medical staff and the use of more sensitive diagnostic tests [4,5].

Laboratory diagnostics play a central role in the detection of acute and chronic HEV infections and provide information on the spread of HEV[6]. According to the guidelines of the European Association for the Study of the Liver, a combination of specific antibody and viral genome detection is recommended [7]. While HEV RNA

can be detected very early in the acute course of infection, the detection of HEV IgM and IgG antibodies provides information on acute and convalescent infections as well as seroprevalence. In immunocompromised patients, reverse-transcription polymerase chain reaction (PCR)-based (quantitative) detection of HEV RNA is essential, as antibodies are sometimes not measurable[7].

With few exceptions, most of the available tests for the detection of HEV antibodies are performed manually in enzyme-linked immunoassay (ELISA) format [8]. DiaSorin has recently launched a fully automated high-throughput test for the detection of anti-HEV IgM and IgG antibodies [9]. The aim of this study was to evaluate the performance of the new Liaison® MUREX anti-HEV IgG and IgM assays in comparison to the established and widely used*recom* Well/*recom* Line HEV IgM and IgG ELISAs/immunoblots from Mikrogen. To our knowledge, there is no data on this yet.

Material and Methods

Samples

The study was performed on 100 pretested HEV IgG-positive, 53 HEV IgM-positive, and 49 samples in which HEV IgG and HEV IgM were undetectable. In addition, 17 samples with serological evidence of acute Epstein-Barr virus (EBV) infection and two samples with acute human cytomegalovirus (CMV) infection were included to investigate possible cross-reactivity. All sera were residual samples which, with the exception of the 19 samples mentioned above, were sent to the laboratory Dr. Krause und Kollegen MVZ GmbH Kiel for serodiagnosis of HEV infection. Information on clinical symptoms as well as liver function was not available to us.

HEV assays

Initial HEV antibody status was determined manually using the*recom* Well HEV IgG or HEV IgM ELISA (Mikrogen GmbH, Neuried, Germany) on a BEP2000 system (Siemens Healthineers AG, Erlangen, Germany); this assay served as a reference here. Sera in which HEV IgG or IgM were detected in this test were immunoblotted (*recom* Line HEV IgG/IgM on a Dynablot Plus system, Mikrogen). The strips were automatically scored (BLOTrix Reader and *recom* Scan software, Mikrogen). The combination of both tests recommended by the manufacturer served as a second reference here.

Subsequently, the sera were re-tested with the fully automated chemiluminescence immunoassay (CLIA) LIAISON® Murex anti-HEV IgM (qualitative) and LIAISON® Murex anti-HEV IgG (quantitative) (DiaSorin Italia S.p.A., Saluggia, Italy).

Sera with evidence of acute EBV infection (N=17; i.e., presence of anti-viral capsid antigen (VCA) IgG/IgM and absence of anti-Epstein–Barr nuclear antigen (EBNA)-1 IgG; Alinity i EBV VCA IgG, EBV VCA IgM and EBV EBNA-1 IgG Reagent Kits, Abbott, Wiesbaden, Germany) or acute/reactivated CMV infection (N=2; i.e., presence of CMV IgM; Abbott Alinity i CMV IgM/IgG Reagent Kit, Abbott) were also analyzed with the HEV antibody assays from Mikrogen and DiaSorin.

Samples with discrepant results were followed up with the WANTAI HEV-IgM and WANTAI HEV-IgG ELISAs (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China), which are known to have particularly high assay sensitivity and specificity [10-12]. It was assumed that the results obtained with two of the three assays were correct. Samples with discrepant results were also tested for the presence of HEV RNA using the RealStar(r) HEV RT-PCR Kit 2.0 (altona Diagnostics GmbH, Hamburg, Germany).

All assays are CE-certified for HEV diagnostics and were used according to the manufacturer's instructions.

The sensitivity and specificity of the DiaSorin tests were determined by help of a four-field table in comparison with the reference method. The 95% confidence interval (CI) was calculated using the freely available software

Graph-Pad (https://www.graphpad.com/quickcalcs/) applying the adjusted Wald method [13].

Results

Method comparison

HEV IgG

The Mikrogen *recom* Well HEV IgG and the LIAISON Murex anti-HEV IgG immunoassays were compared by analyzing 113 blood samples (either IgM, IgG positive/borderline or IgG and IgM positive/borderline in the Mikrogen test) and 49 IgG and IgM negative samples. Table 1 shows the 100% qualitative agreement between the results of both assays. The sensitivity and the specificity is 100 % [95% CI, 0.96 to 1.00] and 100 % [95% CI, 0.91 to 1.00], respectively. In addition, Table 1 demonstrates the qualitative agreement including the immunoblot results. Again, the sensitivity is 100 % [95% CI, 0.96 to 1.00]. No specificity could be determined as no negative samples confirmed by immunoblot were included.

Table 1 – Qualitative agreement of the recomWell/recomLine HEV IgG and the LIAISON(r) Murex anti-HEV IgG immunoassays. The raw data can be found in the supplement. In the recomWell HEV IgG test, a borderline result means that the antibody concentration is in the range of 20 to 24 IU/ml.

		Reference	**Reference**	
		*recom*Well HEV IgG Positive/Borderline	*recom*Well HEV IgG Negative	Total
LIAISON® Murex anti-HEV IgG	Positive	97/3	0	100
	Negative	0	49	49
	Total	100	49	149
		*recom*Well HEV IgG & *recom*Line HEV IgG Positive	*recom*Well HEV IgG & *recom*Line HEV IgG Negative	Total
LIAISON® Murex anti-HEV IgG	Positive	100	0	100
	Negative	0	0	0
	Total	100	0	100

Linearity was demonstrated for three samples in which a very high IgG concentration of approximately 80 U/ml was detected in the*recom* Well HEV IgG assay. These samples were serially diluted in HEV IgG negative serum and measured comparatively in duplicate using the*recom* Well IgG and the LIAISON® Murex anti-HEV IgG assay (Figure 1).

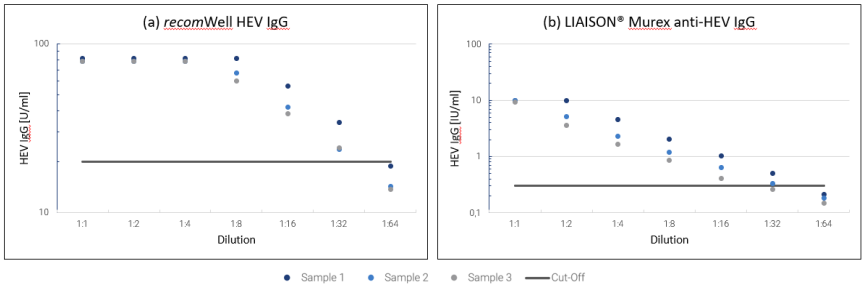

Figure 1 - Linearity of HEV IgG determination over multiple dilution levels. Three samples in which HEV IgG was detectable at high levels were serially diluted in HEV IgG negative serum and measured in duplicate. The 1:64 dilution proved negative in both the recomWell HEV IgG assay (cut-off 20 U/ml) (a) and the LIAISON® Murex anti-HEV IgG assay (cut-off 0.3 IU/ml) (b). The 1:16 dilution level was consistently found to be positive in the recomLine HEV IgG assay, while at the 1:32 dilution level only one of the three sera was positive in the immunoblot. The raw data can be found in the supplement.

HEV-IgM

The *recom* Well HEV IgM and the LIAISON® Murex anti-HEV IgM immunoassays were compared by analyzing 102 serum samples. A sensitivity of 67.9 % [95% CI, 0.54 to 0.79] and a specificity of 100% [95% CI, 0.91 to 1.00] was calculated (Table 2). An aberrant result was demonstrated in 17 samples, so the WANTAI HEV IgM immunoassay was used to decide how to score these sera. Nine out of 17 samples were quoted HEV IgM negative by the WANTAI assay and confirmed the result of the LIAISON® Murex IgM CLIA (Supplementary data). Thus, a sensitivity of 81.8% [95%CI, 0.68 to 0.91] and a specificity of 100% [95% CI, 0.91 to 1.00] was calculated for the LIAISON test. When immunoblot results were considered, the sensitivity and specificity of the latter was 88.9 % [95% CI, 0.71 to 0.97] and 53.9 % [95% CI, 0.35 to 0.71], respectively (Table 2). HEV RNA was not detected in any of the 17 discrepant sera by RT-PCR.

Table 2 – Agreement of the recomWell/recomLine HEV IgM and DiaSorin LIAISON® Murex anti-HEV IgM immunoassays. The raw data can be found in the supplement.* HEV RNA was not detected in these 17 samples by RT-PCR. In the recomWell HEV IgM test, a borderline result means that the antibody concentration is in the range of 20 to 24 IU/ml.

		Reference	**Reference**	
		*recom*Well HEV IgM Positive/Borderline	*recom*Well HEV IgM Negative	Total
LIAISON® Murex anti-HEV IgM	Positive	35/1	0	36
	Negative	13/4*	49	66
	Total	53	49	102
		*recom*Well HEV IgM & *recom*Line HEV IgM Positive	*recom*Well HEV IgM & *recom*Line HEV IgM Negative	Total
LIAISON® Murex anti-HEV IgM	Positive	24	12	36
	Negative	3	14	17
	Total	27	26	53

Exclusion of HEV antibody cross-reactivity due to polyclonal stimulation

A total of 17 samples with signs of acute EBV infection and two samples with evidence of CMV infection were re-tested for HEV IgG/IgM. The same three acute EBV samples were identified as HEV IgG positive in all tests. All tests detected one common acute EBV serum positive for HEV IgM. In addition, two different acute EBV sera were identified as HEV IgM carriers using the Mikrogen and DiaSorin assays. DiaSorin detected one sample with isolated HEV IgM. For the two CMV sera, the Mikrogen and DiaSorin tests gave identical results (Table 3).

Table 3 – Samples with evidence for an acute Epstein-Barr Virus (EBV) or Human Cytomegalovirus (CMV) infection were tested for the presence of HEV antibodies. In sample no. 2, isolated IgM (without HEV IgG) was detected with one test and therefore quoted as false reactive. The measured raw values are given in brackets. * This sample should be quoted as borderline in the recomWell HEV IgM test ([?]20 U/ml and [?]24 U/ml). Abbreviations: +, positive; -, negative; n.t., not tested.

No.	Infect.	HEV IgG	HEV IgG	HEV IgG	HEV IgG	HEV IgM	HEV IgM	HEV IgM	HEV IgM
		Mikrogen *recom*Well	Mikrogen *recom*Line	DiaSorin	Wantai	Mikrogen *recom*Well	Mikrogen *recom*Line	DiaSorin	Wantai
		qual. (U/ml)	qual.	qual. (IU/ml)	qual. (S/CO)	qual. (U/ml)	qual.	qual. (Index)	qual. (S/CO)
1	EBV	+(>125)	+	+(>10)	+(15.8)	+(31.6)	-	+(1.1)	+(5.5)
2	EBV	-(3.0)	n.t.	-(<0.1)	-(0.0)	-(9.0)	n.t.	+(1.6)	-(0.1)
3	EBV	-(2.0)	n.t.	-(<0.1)	-(0.0)	-(4.5)	n.t.	-(0.2)	-(0.2)
4	EBV	-(3.9)	n.t.	-(<0.1)	-(0.0)	-(1.9)	n.t.	-(<0.1)	-(0.0)
5	EBV	-(3.1)	n.t.	-(<0.1)	-(0.0)	-(3.0)	n.t.	-(0.2)	-(0.0)
6	EBV	-(2.1)	n.t.	-(<0.1)	-(0.0)	-(3.0)	n.t.	-(0.1)	-(0.1)
7	EBV	+(50.3)	+	+(1.1)	+(12.8)	-(13.4)	n.t.	-(0.5)	-(0.6)
8	EBV	+(44.8)	+	+(1.4)	+(10.8)	+(20.4)*	-	-(0.6)	-(0.0)
9	EBV	-(3.7)	n.t.	-(<0.1)	-(0.0)	-(2.6)	n.t.	-(0.2)	-(0.0)
10	EBV	-(3.0)	n.t.	-(<0.1)	-(0.0)	-(7.3)	n.t.	-(0.2)	-(0.0)
11	EBV	-(5.4)	n.t.	-(<0.1)	-(0.0)	-(4.5)	n.t.	-(0.9)	-(0.0)
12	EBV	-(3.6)	n.t.	-(<0.1)	-(0.0)	-(6.7)	n.t.	-(0.2)	-(0.0)
13	EBV	-(3.0)	n.t.	-(<0.1)	-(0.0)	-(5.1)	n.t.	-(0.6)	-(0.1)
14	EBV	-(4.0)	n.t.	-(<0.1)	-(0.0)	-(2.1)	n.t.	-(0.1)	-(0.1)
15	EBV	-(2.7)	n.t.	-(<0.1)	-(0.0)	-(2.7)	n.t.	-(0.7)	-(0.0)
16	EBV	-(5.1)	n.t.	-(<0.1)	-(0.0)	-(7.7)	n.t.	-(0.1)	-(0.0)
17	EBV	-(3.4)	n.t.	-(<0.1)	-(0.0)	-(6.7)	n.t.	-(<0.1)	-(0.0)
18	CMV	+(39.5)	n.t.	+(0.5)	n.t.	- (16.9)	n.t.	-(0.5)	n.t.
19	CMV	-(6.9)	n.t.	-(<0.1)	-(0.0)	- (11.2)	n.t.	-(0.2)	-(0.0)

Discussion

Immunoassays for the detection of HEV IgM and IgG antibodies are widely used because of their ease of use and comparatively low cost. The problem, however, is that the tests have different sensitivity and specificity and give qualitative, semi-quantitative or quantitative results [8,11,12,14-16]. The differential performance of anti-HEV IgG assays has important implications for seroprevalence estimates [17]. A WHO reference serum (NISBSC 95/584) for standardizing HEV antibody tests has been available for several years [18] and could

help to improve assay comparability.

In this study, the LIAISON Murex anti-HEV IgG CLIA showed consistent results with the *recom* Well HEV IgG ELISA used as a reference. These assays provide quantitative results. The LIAISON Murex Anti-HEV IgG test is aligned with the WHO standard. In general, both assays appear to be suitable for seroprevalence studies. The CLIA has the advantage of being fully automated.

HEV IgM antibody test results are more heterogeneous and require detailed discussion. The highest number of HEV IgM-positive samples was found with the *recom* Well HEV ELISA. However, HEV RNA could not be detected in any of the 17 samples that were reactive in this test but not in the DiaSorin assay. Therefore, a post-acute infection status, persistent IgM or even a false-positive IgM detection is assumed. If the*recom* Well HEV IgM ELISA is used in combination with the HEV IgM*recom* Line immunoblot, as recommended by the manufacturer, the number of IgM detections is reduced about almost 50% (27 out of 53 positive samples are confirmed by immunoblot). Eight of 17 samples found to be reactive in the *recom* Well HEV IgM ELISA were concordantly negative in the *recom* Line HEV IgM blot and in the Diasorin and Wantai IgM assays (supplementary material). Recently, good agreement was reported between HEV antibody tests from the latter two manufacturers[9].

In four of the 53 IgM-positive sera, no corresponding IgG antibodies were detectable (quoted as isolated HEV IgM). Two of these samples were reactive in the *recom* Well HEV IgM ELISA, the *recom* Line HEV IgM immunoblot and the CLIA, while two samples were reactive only in the*recom* Well HEV IgM test (supplementary material). It is suspected that these two samples were false positive for HEV IgM. This finding underlines the importance of the *recom* Line HEV IgM immunoblot for the verification of reactive ELISA results. In general, detection of isolated HEV IgM should prompt confirmatory and follow-up testing.

The investigation of a limited number of samples with serologically suspected acute EBV/CMV infection revealed possible HEV-IgM cross-reactivity confirming the results of a previous study[19]. This phenomenon is most likely due to polyclonal B-cell stimulation associated with herpesvirus infection[19]. Therefore, patients with isolated HEV IgM should be followed up after a few weeks. If necessary, EBV and CMV serostatus should also be determined.

Conclusion

The fully-automated LIAISON® Murex anti-HEV IgG and IgM assays are sensitive and specific high-throughput tests with good performance. Both tests can be used for the diagnosis of acute and convalescent HEV infections. The HEV IgG CLIA is also suitable for seroprevalence studies.

Declarations

Ethics approval and consent to participate

The study was conducted according to the principles of the Declaration of Helsinki and registered with the Ethics Committee of the Medical Faculty of Kiel University (D585/21).

Consent for publication

All authors have read the final manuscript and agree to publication.

Availability of data and materials

All data can be found in the manuscript or its supplement.

Competing interests

The authors have no conflicts of interest. DiaSorin played an important role in the preparation of the publication. The company supported this study by providing test kits and a financial grant. The first author, A.E., is employed by DiaSorin. This company had no influence on the selection of the samples, their testing and the interpretation of the results. The results of the tests were also communicated to Mikrogen GmbH.

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The costs of the study were covered by DiaSorin.

Authors' contributions

AE, AK and FN have planned the study. CB, FN, FS, IG and SM have tested the samples and collected the data. AK and FN have evaluated the data. AE, AK and FN wrote the manuscript. All authors have read the manuscript.

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References

1. Wang B, Meng XJ. Hepatitis E virus: host tropism and zoonotic infection. *Curr Opin Microbiol.* 2021;59:8-15.

2. Denner J. Hepatitis E virus (HEV)-The Future. *Viruses.*2019;11(3).

3. Denner J, Pischke S, Steinmann E, Blumel J, Glebe D. Why all blood donations should be tested for hepatitis E virus (HEV). *BMC Infect Dis.* 2019;19(1):541.

4. Dudareva S, Faber M, Zimmermann R, et al. [Epidemiology of viral hepatitis A to E in Germany]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2022;65(2):149-158.

5. Adlhoch C, Avellon A, Baylis SA, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. *J Clin Virol.* 2016;82:9-16.

6. Zhao C, Wang Y. Laboratory Diagnosis of HEV Infection. *Adv Exp Med Biol.* 2016;948:191-209.

7. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines on hepatitis E virus infection. *J Hepatol.*2018;68(6):1256-1271.

8. Abravanel F, Goutagny N, Joffray R, et al. Performance characteristics of the VIDAS(R) ANTI-HEV IgM and IgG assays. *J Clin Virol.* 2019;112:10-14.

9. Abravanel F, Parraud D, Chapuy-Regaud S, et al. Diagnostic Performance of an Automated System for Assaying Anti-Hepatitis E Virus Immunoglobulins M and G Compared with a Conventional Microplate Assay. *Viruses.* 2022;14(5).

10. Kmush BL, Labrique AB, Dalton HR, et al. Two Generations of "Gold Standards": The Impact of a Decade in Hepatitis E Virus Testing Innovation on Population Seroprevalence. *Am J Trop Med Hyg.*2015;93(4):714-717.

11. Pas SD, Streefkerk RH, Pronk M, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. *J Clin Virol.* 2013;58(4):629-634.

12. Sommerkorn FM, Schauer B, Schreiner T, Fickenscher H, Krumbholz A. Performance of Hepatitis E Virus (HEV)-antibody tests: a comparative analysis based on samples from individuals with direct contact to domestic pigs or wild boar in Germany. *Med Microbiol Immunol.*2017;206(3):277-286.

13. Agresti A, Coull BA. Approximate is Better than "Exact" for Interval Estimation of Binomial Proportions. *The American Statistician.* 1998;52(2):119-126.

14. Dichtl K, Zimmermann J, Koeppel MB, Bohm S, Osterman A. Evaluation of a Novel CLIA Monotest Assay for the Detection of Anti-Hepatitis E Virus-IgG and IgM: A Retrospective Comparison with a Line Blot and an ELISA. *Pathogens.* 2021;10(6).

15. Shrestha AC, Flower RL, Seed CR, Stramer SL, Faddy HM. A Comparative Study of Assay Performance of Commercial Hepatitis E Virus Enzyme-Linked Immunosorbent Assay Kits in Australian Blood Donor Samples. *J Blood Transfus.* 2016;2016:9647675.

16. Vollmer T, Diekmann J, Eberhardt M, Knabbe C, Dreier J. Monitoring of Anti-Hepatitis E Virus Antibody Seroconversion in Asymptomatically Infected Blood Donors: Systematic Comparison of Nine Commercial Anti-HEV IgM and IgG Assays. *Viruses.* 2016;8(8).

17. 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(3):497-500.

18. Ferguson M, Walker D, Mast E, Fields H. Report of a collaborative study to assess the suitability of a reference reagent for antibodies to hepatitis E virus. *Biologicals.* 2002;30(1):43-48.

19. Fogeda M, de Ory F, Avellon A, Echevarria JM. Differential diagnosis of hepatitis E virus, cytomegalovirus and Epstein-Barr virus infection in patients with suspected hepatitis E. *J Clin Virol.*2009;45(3):259-261.

Title: Diagnostic performance of a fully automated hepatitis E virus antibody immunoassay

Running title: Performance of automated HEV antibody tests

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Table 1 – Raw data for the comparison of the Mikrogen and DiaSorin HEV IgG immunoassays. *The cut-off for positive results is 20 U/ml and 0.3 IU/ml for Mikrogen recomWell IgG and DiaSorin LIAISON® Murex anti-HEV IgG, respectively. * These samples should be quoted as borderline in the recomWell HEV IgG test (≥20 U/ml and ≤24 U/ml). Abbreviations: qual., qualitative; +, positive.*

Number	**Mikrogen** *recom*Well HEV IgG		**Mikrogen** *recom*Line HEV IgG	**DiaSorin** LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
1	29.0	+	+	0.5	+
2	80.4	+	+	1.3	+
3	79.7	+	+	2.4	+
4	>125	+	+	>10	+
5	>125	+	+	>10	+
6	113.8	+	+	4.5	+
7	>125	+	+	3.7	+
8	>125	+	+	>10	+
9	89.3	+	+	2.4	+
10	103.2	+	+	>10	+
11	103.2	+	+	5.4	+
12	103.2	+	+	>10	+
13	71.4	+	+	1.3	+
14	98.8	+	+	2.9	+
15	91.2	+	+	3.4	+
16	80.0	+	+	1.9	+
17	98.8	+	+	>10	+
18	89.4	+	+	3.1	+
19	35.1	+	+	0.8	+
20	92.3	+	+	>10	+
21	25.4	+	+	0.5	+
22	92.3	+	+	>10	+
23	183.2	+	+	>10	+
24	40.7	+	+	0.6	+
25	59.5	+	+	1.1	+
26	54.3	+	+	0.8	+
27	69.4	+	+	1.1	+
28	107.0	+	+	>10	+
29	107.0	+	+	>10	+
30	96.0	+	+	3.6	+
31	26.1	+	+	0.7	+
32	107.0	+	+	4.7	+

Number	**Mikrogen** *recom*Well HEV IgG		**Mikrogen** *recom*Line HEV IgG	**DiaSorin** LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
33	107.0	+	+	>10	+
34	117.5	+	+	3.8	+
35	117.5	+	+	>10	+
36	71.7	+	+	1.3	+
37	82.3	+	+	1.5	+
38	32.1	+	+	0.5	+
39	98.4	+	+	6.3	+
40	98.4	+	+	>10	+
41	34.4	+	+	0.49	+
42	40.9	+	+	1.5	+
43	79.4	+	+	1.2	+
44	98.4	+	+	3.5	+
45	46.2	+	+	0.8	+
46	96.4	+	+	4.4	+
47	96.4	+	+	>10	+
48	41.2	+	+	0.7	+
49	41.9	+	+	0.8	+
50	31.0	+	+	0.5	+
51	74.4	+	+	1.6	+
52	92.4	+	+	5.9	+
53	27.2	+	+	0.6	+
54	28.1	+	+	0.5	+
55	51.3	+	+	1.1	+
56	97.6	+	+	>10	+
57	97.6	+	+	>10	+
58	97.6	+	+	>10	+
59	23.9*	+	+	0.4	+
60	>125	+	+	>10	+
61	>125	+	+	6.5	+
62	23.9*	+	+	0.3	+
63	44.7	+	+	0.8	+
64	73.0	+	+	1.6	+
65	>125	+	+	>10	+
66	>125	+	+	>10	+
67	101.7	+	+	7.2	+
68	101.7	+	+	3.6	+
69	21.2*	+	+	0.3	+
70	>125	+	+	>10	+
71	>125	+	+	>10	+
72	>125	+	+	>10	+
73	36.1	+	+	0.9	+
74	60.2	+	+	1.5	+
75	49.1	+	+	0.8	+
76	85.5	+	+	1.9	+
77	106.7	+	+	>10	+
78	34.2	+	+	0.6	+
79	86.4	+	+	2.6	+
80	122.7	+	+	8.5	+
81	34.6	+	+	0.6	+
82	35.7	+	+	0.5	+
83	73.3	+	+	1.6	+
84	25.4	+	+	0.4	+
85	>125	+	+	>10	+
86	>125	+	+	5.0	+
87	>125	+	+	>10	+
88	103.2	+	+	9.8	+

Number	**Mikrogen** *recom*Well HEV IgG		**Mikrogen** *recom*Line HEV IgG	**DiaSorin** LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
89	98.8	+	+	>10	+
90	92.3	+	+	>10	+
91	43.0	+	+	0.7	+
92	38.5	+	+	0.4	+
93	37.8	+	+	0.4	+
94	>125	+	+	>10	+
95	58.5	+	+	1.3	+
96	>125	+	+	5.2	+
97	63.9	+	+	1.4	+
98	84.7	+	+	2.0	+
99	106.7	+	+	>10	+
100	66.3	+	+	1.4	+

Table 2 - Raw data for the comparison of the Mikrogen, DiaSorin and Wantai HEV IgM immunoassays. *The cut-off for positive results is 20 U/ml and 1 [Index] for Mikrogen recomWell HEV IgG and DiaSorin LIASON® Murex anti-HEV IgM, respectively. Only sera that had a discrepant result between the recomWell HEV IgM ELISA and the DiaSorin assay were re-screened in the Wantai HEV IgM assay (positive = absorbance value of the sample/absorbance value of the cut-off ≥1) . * These samples should be quoted as borderline in the recomWell HEV IgM test (≥20 U/ml and ≤24 U/ml). Abbreviations: qual., qualitative; +, positive; -, negative; n.t., not tested).*

Number	**Mikrogen** *recom*Well HEV IgM		**Mikrogen** *recom*Line HEV IgM	**DiaSorin** LIAISON® Murex anti-HEV IgM		**Wantai** HEV-IgM ELISA		**RealStar®** HEV RT-PCR Kit 2.0	**Mikrogen** *recom*Well HEV IgG	
	[U/ml]	qual.	qual.	[Index]	qual.	sample/ cut-off	qual.	RNA	[U/ml]	qual.
1	114.5	+	+	3.1	+	n.t.	n.t.	n.t.	85.2	+
2	83.7	+	-	2.8	+	n.t.	n.t.	n.t.	85.2	+
3	>125	+	-	2.9	+	n.t.	n.t.	n.t.	>125	+
4	100.7	+	+	1.7	+	n.t.	n.t.	n.t.	98.7	+
5	35.6	+	-	0.5	-	0.29	-	-	98.8	+
6	34.5	+	-	0.8	-	0.44	-	-	98.8	+
7	68.7	+	+	3.3	+	n.t.	n.t.	n.t.	91.2	+
8	43.4	+	-	0.6	-	3.98	+	-	98.8	+
9	24.4	+	-	0.8	-	1.2	+	-	103.2	+
10	33.7	+	-	1.3	+	n.t.	n.t.	n.t.	89.3	+
11	45.4	+	-	1.1	+	n.t.	n.t.	n.t.	>125	+
12	>125	+	+	9.1	+	n.t.	n.t.	n.t.	>125	+
13	21.1*	+	+	0.97	-	2.6	+	-	>125	+
14	>125	+	+	9.1	+	n.t.	n.t.	n.t.	>125	+
15	39.7	+	+	1.6	+	n.t.	n.t.	n.t.	79.7	+
16	93.4	+	+	5.4	+	n.t.	n.t.	n.t.	92.3	+
17	187.5	+	+	6.9	+	n.t.	n.t.	n.t.	183.2	+
18	98.4	+	+	1.7	+	n.t.	n.t.	n.t.	117.5	+
19	57.5	+	-	1.9	+	n.t.	n.t.	n.t.	117.5	+
20	35.7	+	+	1.5	+	n.t.	n.t.	n.t.	14.7	-
21	28.7	+	+	0.99	-	1.08	(+)	-	96.0	+
22	>125	+	+	4.0	+	n.t.	n.t.	n.t.	107.0	+
23	72.8	+	-	2.2	+	n.t.	n.t.	n.t.	107.0	+
24	34.2	+	-	1.4	+	n.t.	n.t.	n.t.	107.0	+
25	22.1*	+	-	0.8	-	1.68	+	-	107.0	+
26	28.7	+	-	<0.1	-	0.03	-	-	4.0	-
27	83.3	+	+	3.2	+	n.t.	n.t.	n.t.	96.4	+
28	24.2	+	-	<0.1	-	0.02	-	-	2.3	-
29	120.1	+	+	4.6	+	n.t.	n.t.	n.t.	96.4	+
30	23.3*	+	+	0.6	-	0.22	-	-	32.1	+

Number	**Mikrogen** *recom*Well HEV IgM		**Mikrogen** *recom*Line HEV IgM	**DiaSorin** LIAISON® Murex anti-HEV IgM		**Wantai** HEV-IgM ELISA		**RealStar®** HEV RT-PCR Kit 2.0	**Mikrogen** *recom*Well HEV IgG	
	[U/ml]	qual.	qual.	[Index]	qual.	sample/ cut-off	qual.	RNA	[U/ml]	qual.
31	116.7	+	+	5.9	+	n.t.	n.t.	n.t.	98.4	+
32	21.1*	+	-	0.6	-	0.06	-	-	92.4	+
33	27.3	+	-	1.8	+	n.t.	n.t.	n.t.	51.3	+
34	104.4	+	+	1.6	+	n.t.	n.t.	n.t.	92.4	+
35	33.3	+	+	1.4	+	n.t.	n.t.	n.t.	51.3	+
36	46.3	+	-	1.1	+	n.t.	n.t.	n.t.	97.6	+
37	43.2	+	-	1.0	+	n.t.	n.t.	n.t.	>125	+
38	>125	+	+	8.7	+	n.t.	n.t.	n.t.	27.5	+
39	37.5	+	-	0.8	-	1.66	+	-	>125	+
40	22.3*	+	+	1.8	+	n.t.	n.t.	n.t.	3	-
41	>125	+	+	6.6	+	n.t.	n.t.	n.t.	>125	+
42	>125	+	+	3.0	+	n.t.	n.t.	n.t.	>125	+
43	24.5	+	-	0.8	-	0.02	-	-	63.9	+
44	30.5	+	-	0.6	-	1.18	+	-	101.7	+
45	52.0	+	-	1.1	+	n.t.	n.t.	n.t.	101.7	+
46	>125	+	+	7.0	+	n.t.	n.t.	n.t.	>125	+
47	43.0	+	-	1.4	+	n.t.	n.t.	n.t.	>125	+
48	25.1	+	-	0.7	-	0.68	-	-	85.5	+
49	27.0	+	-	0.7	-	0.02	-	-	49.1	+
50	66.1	+	+	2.2	+	n.t.	n.t.	n.t.	106.7	+
51	31.4	+	-	0.9	-	1.12	+	-	106.7	+
52	>125	+	+	8.2	+	n.t.	n.t.	n.t.	122.7	+
53	>125	+	+	2.2	+	n.t.	n.t.	n.t.	>125	+

Table 3 – Raw data of the HEV antibody negative samples included in the study.

Number	**Mikrogen** *recom*Well HEV IgG	**DiaSorin** LIAISON® Murex anti-HEV IgG	**Mikrogen** *recom*Well HEV IgM	**DiaSorin** LIAISON® Murex anti-HEV IgM
	[U/ml]	[IU/ml]	[U/ml]	[Index]
1	1.9	<0.1	3.1	<0.1
2	1.5	0.2	2.8	<0.1
3	5.2	<0.1	3.5	<0.1
4	9.2	0.3	2.8	0.2
5	1.5	<0.1	3.8	0.1
6	2.6	<0.1	7.8	<0.1
7	3.4	<0.1	4.3	0.1
8	2.5	<0.1	2.2	<0.1
9	2.1	<0.1	3.0	0.4
10	2.1	<0.1	10.6	<0.1
11	2.7	<0.1	2.2	<0.1
12	2.5	<0.1	5.4	<0.1
13	2.2	<0.1	1.5	<0.1
14	10.3	0.1	5.4	0.2
15	2.8	<0.1	2.0	<0.1
16	3.4	<0.1	1.5	<0.1
17	2.9	<0.1	2.5	<0.1
18	1.7	<0.1	7.9	0.3
19	2.6	<0.1	3.2	<0.1
20	1.9	<0.1	2.5	<0.1
21	8.8	0.1	2.7	<0.1
22	1.8	<0.1	1.9	<0.1
23	1.2	<0.1	1.7	<0.1
24	2.0	<0.1	2.5	<0.1
25	2.2	<0.1	1.8	<0.1
26	1.2	<0.1	2.1	<0.1
27	1.3	<0.1	2.0	<0.1
28	2.2	<0.1	2.4	<0.1
29	1.7	<0.1	2.8	<0.1
30	14.6	0.2	2.1	<0.1
31	11.2	0.1	2.7	<0.1
32	12.0	0.2	2.3	<0.1
33	19.2	0.2	1.8	<0.1
34	2.0	<0.1	2.4	<0.1
35	4.5	<0.1	4.6	<0.1
36	9.9	<0.1	8.2	<0.1
37	5.4	<0.1	4.3	<0.1
38	5.1	0.2	2.6	<0.1
39	6.1	<0.1	4.4	<0.1
40	3.6	<0.1	6.7	0.8
41	2.3	<0.1	7.9	0.4
42	3.6	<0.1	5.8	<0.1
43	5.5	<0.1	4.4	0.1
44	3.4	<0.1	2.3	<0.1
45	4.3	<0.1	5.7	<0.1
46	3.2	<0.1	4.3	0.3
47	3.9	<0.1	2.4	<0.1
48	5.0	0.1	5.2	0.1
49	3.1	<0.1	3.4	<0.1

Table 4 – Raw data of the HEV IgG linearity studies. *For all three investigated samples individual as well as mean values are given. The cut-off for positive results is 20 U/ml and 0.3 IU/ml for Mikrogen recomWell HEV IgG and DiaSorin LIAISON® Murex anti-HEV IgG, respectively. * These measurements should be quoted as borderline in the recomWell HEV IgG test (≥20 U/ml and ≤24 U/ml). Some sera dilutions around the cut-off of the recomWell HEV IgG test were also re-analyzed by immunoblot. Abbreviations: +, positive; -, negative; n.t., not tested.*

Sample	**Dilution**	**Mikrogen** recomWell HEV IgG		**Mikrogen** recomLine HEV IgG	**DiaSorin** Murex anti-HEV IgG	
		[U/ml]	Mean [U/ml]		[IU/ml]	Mean [IU/ml]
# 1	1:1	81.9	81.9	n.t.	>10	>10
		81.9			>10	
	1:2	81.9	81.9	n.t.	9.5	>10
		81.9			>10	
	1:4	81.9	81.9	n.t.	4.6	4.6
		81.9			4.6	
	1:8	81.9	81.9	n.t.	2.0	2.0
		81.9			2.0	
	1:16	57.7	56.4	+	1.0	1.0
		55.0			1.0	
	1:32	34.2	34.1	-	0.5	0.5
		34.1			0.5	
	1:64	19.6	18.9	-	0.2	0.2
		18.2			0.2	
# 2	1:1	79.0	79.0	n.t.	>10	>10
		79.0			>10	
	1:2	79.0	79.0	n.t.	4.7	5.1
		79.0			5.5	
	1:4	79.0	79.0	n.t.	2.3	2.3
		79.0			2.3	
	1:8	65.9	66.9	n.t.	1.1	1.2
		67.8			1.2	
	1:16	44.2	42.0	+	0.6	0.6
		39.7			0.6	
	1:32	24.6	23.6*	+	0.3	0.4
		22.5*			0.4	
	1:64	14.0	14.3	n.t.	0.2	0.2
		14.6			0.2	
# 3	1:1	79.0	79.0	n.t.	9.4	9.2
		79.0			9.1	
	1:2	79.0	79.0	n.t.	3.8	3.6
		79.0			3.4	
	1:4	79.0	79.0	n.t.	1.7	1.7
		79.0			1.6	
	1:8	61.4	60.0	n.t.	0.9	0.9
		58.7			0.8	
	1:16	39.0	38.7	+	0.4	0.4
		38.3			0.4	
	1:32	24.6	24.1	-	0.3	0.3
		23.5*			0.3	
	1:64	13.9	13.7	n.t.	0.1	0.2
		13.5			0.2	