EUROPEAN COMMISSION

View in the context of the Performance Evaluation Consultation Procedure (PECP)

Expert panels on medical devices and in vitro diagnostic devices (Expamed)

Contents

Scope of this expert view

This scientific view reflects the opinion of independent experts (MDR Article 106.1) on the performance evaluation report (PER) of the manufacturer. The advice is provided in the context of the performance evaluation consultation procedure (PECP), which is an additional element of conformity assessment by notified bodies for specific high-risk in vitro diagnostic devices (IVDR Article 48.6).

When making its conformity assessment decision, the notified body is obliged to give due consideration to the opinions expressed in the scientific view of the expert panel, where applicable (Annex IX, Section 4.9 or, as applicable, Annex X, Section 3, point (j)).

For class D devices, the notified body must provide a full justification in the case of divergent views between the notified body and the experts. This justification shall be included in the notification to the competent authority (IVDR Article 50; mechanism for scrutiny of class D devices).

1 ADMINISTRATIVE INFORMATION

Date of reception of the dossier	02/08/2023
Notified Body number	0459
Internal PECP dossier # (e.g. 2021-000201)	IVD-2023-000017
***In vitro* diagnostic medical device** *(descriptive text, no nomenclature use)*	Essay using chemiluminescent immunoassay (CLIA) technology for the quantitative determination of IgG antibodies to hepatitis E virus (anti-HEV IgG) in human serum and plasma samples included specimens collected postmortem (non-heart beating). It is intended as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis and to evaluate the presence and the amount of anti-HEV IgG. It is also intended as a screening test for organ, tissue, and cells postmortem donors.

2 INFORMATION PROVIDED BY THE NOTIFIED BODY

When consulting the IVD expert panel, the notified body provided the below information on the type of device in accordance with MDCG 2021-22.

Intended purpose (P)		
P1	what is detected and/or measured *please specify the analyte(s) or marker(s), e.g. SARS-CoV-2 spike protein, Kel1 (K)*	Anti-HEV IgG
P2	function of the device *e.g. diagnosis, aid to diagnosis, monitoring, determining the infectious load, tissue typing etc*	Aid in the diagnosis of HEV infection. Screening test for post-mortem organ, tissue and cells donors.
P3	the specific disorder, condition or risk factor of interest that it is intended to detect, define or differentiate *e.g. hepatitis C infection, exposure to SARS-CoV-2, risk of HIV transmission in blood transfusion etc.*	HEV infection
P4	whether it is automated or not	Automated assay

P5	whether it is qualitative, semi-quantitative or quantitative	Qualitative assay
P6	type of specimen(s) *e.g. whole blood, serum, saliva etc*	Serum and plasma samples, including specimens collected post-mortem (non-heart beating).
P7	where applicable, the testing population *e.g. persons with specific health conditions, persons with specific symptoms, children in a certain age range*	Individuals with or without symptoms of hepatitis.
P8	intended user	For Laboratory Professional Use Only
Technology (T)		
T1	principle of the assay method or principles of operation of the instrument *e.g. real-time PCR, qualitative PCR, digital PCR, sandwich immunoassay, competitive immunoassay, immunoturbidimetric assay etc.*	Chemiluminescence immunoassay (CLIA).

3 VIEWS OF THE EXPERT PANEL

3.1 Information on panel and sub-group (where relevant)

Date of views	19/10/2023
Expert panel name	IVD expert panel
Sub-group of expert panel (where relevant)	IVD sub-group 2023-17

3.2 Summary of expert panel views

Hepatitis E is a liver infection caused by the hepatitis E virus (HEV) characterized by a single-stranded RNA genome. The diagnosis of Hepatitis E can only be confirmed by testing for IgM antibodies against HEV or HEV RNA because there is no clinical difference between these two markers for acute viral hepatitis. A sample potentially infectious for HEV can optimally be determined by detection of HEV RNA after nucleic acid amplification, e.g. by PCR. Presence of anti-HEV IgM at the early infection phase may overlap with some parts of HEV viraemia.

This anti-HEV IgG device is a chemiluminescent immunoassay (CLIA) assay for the quantitative determination of IgG antibodies to hepatitis E virus (anti-HEV IgG) in human serum and plasma samples, including specimens collected postmortem (non-heart beating). The assay is intended to aid in diagnosing HEV infection in individuals with or without symptoms of hepatitis and to evaluate the presence and the amount of anti-HEV IgG. However, the assay can only be used to determine if an infection with HEV has occurred in the past, or if an HEV vaccination has been applied.

The assay's intended use specified by the manufacturer is to screen human donor samples for anti-HEV IgG from post-mortem donors, with plasma or serum being screened as individual samples. The assay is carried out in a closed equipment provided by the manufacturer.

Although anti-HEV IgG is recognized as a marker of past infection (European Association for the Study of the Liver (EASL) - Clinical Practice Guidelines on hepatitis E virus infection, Journal of Hepatology 2018;68(6):1256.1271 DOI:https://doi.org/10.1016/j.jhep.2018.03.005), the manufacturer states that its intended use is also "valuable for the diagnosis of HEV infection" and "as a screening test for postmortem organ, tissue, and cell donors." (see Instructions for Use, 1. Intended Purpose; Scientific Validity Report 8.1 and 8.2). This claim suggests rather a detection of acute infection than an indication of resolved infections. However, this is not the accepted view of the scientific literature for the diagnostic marker anti-HEV IgG.

The intended use/fitness for purpose of the anti-IgG HEV assay binary proprieties (positive/negative) is to determine whether an individual has been in contact with the HEV virus. However, it cannot be used as a screening assay alone for the determination of an acute infection with HEV. In those cases, additional testing with anti-IgM antibodies and the detection of HEV RNA is necessary as recommended by the HEV guidelines (EASL Clinical Practice Guidelines on hepatitis E virus infection).

Finally, and due to its technical characteristics and the biological marker detected, it is this Expert Panel's view that this device should not be used to detect the presence of, or exposure to, HEV in blood, blood components, cells, tissues or organs, or in any of their derivatives, in order to assess their suitability for transfusion, transplantation or cell administration because anti-HEV IgG is considered a marker of past resolved infection characterized by absence of HEV viraemia.

3.3 Views on the specific reports included in the performance evaluation report (PER)

(IVDR, Annex XIII, Section 1.3.2, first paragraph)

Views of the expert panel on the performance evaluation report of the manufacturer (PER)
1. Expert views on the scientific validity report[1]
The manufacturer describes range and context in the Scientific Validity Report as "The detection of antibodies anti-HEV provides a marker of exposure to the HEV infection. Acute HEV infection can be diagnosed by the detection of anti-HEV antibodies (IgM, IgG, or both) in combination with HEV RNA." Patients with reinfection are typically anti-IgM HEV negative, but IgG and PCR positive. "Past infection is determined by the presence of anti-HEV IgG". Some authors (Nassim Kamar, Jacques Izopet, Nicole Pavio, Rakesh Aggarwal, Alain Labrique, Heiner Wedemeyer and Harry R. Dalton. Hepatitis E virus infection. Nat Rev Dis Primers. 2017 Nov 16;3:17086. DOI: 10.1038/nrdp.2017.86.) emphasize that determining the anti-HEV IgG concentration could be useful for estimating the risk of reinfection after natural infection or after vaccination in clinical trials. The manufacturer's claims are consistent with the "EASL Clinical Practice Guidelines on Hepatitis E virus infection" (2018) by the European Association for the Study of the Liver. However, the text seems to confuse the IgG antibody as a screening marker, when its interest seems limited to recognizing a past infection, including cases of reinfection with HEV with HEV RNA positivity. The assessment of the claims again seems to ignore the limitation of the HEV IgG antibody for screening. Whether in "Claim 1. Antibody to HEV (IgG and IgM) detection is valuable for the diagnosis of HEV infection" or in "Claim 2. Antibody to HEV (IgG and IgM) detection is valuable for the screening of organ, tissue, and cells postmortem donors." Moreover, in the assessment of Claim 2, IgG is never mentioned, the text being generic. The requirement for HEV screening should not be confused with IgG determination, which seems to happen. Furthermore, it should be mentioned that routine screening for antibodies against HEV, whether IgM and/or IgG, is not a standard procedure for screening postmortem donors.
2. Expert views on the analytical performance report[2]
The performance characteristics tested and accepted the following matrices: serum (without and with gel SST); sodium citrate; sodium and lithium heparin plasma; K2-EDTA; potassium oxalate; CPDA plasma; and ACD plasma. Postmortem specimens, collected up to 24 hours after death, have been tested and may also be used in the assay. Specimen handling, collection, and storage claims have been verified. The following storage conditions showed no significant differences: 15°-30°C for 48 hours. In any case, room temperature storage should be avoided: 2°- 8°C for 48 hours. Otherwise, specimens should be aliquoted and stored deep-frozen (–20°C or below) for up to 2 freeze-thaw cycles. However, multiple freeze-thaw cycles should be avoided. Up to one month at -20°C or below. If samples are stored frozen, mix thawed samples well before testing.

[1] Annex XIII, Section 1.2.1 of Regulation (EU) 2017/746 - Demonstration of the scientific validity

[2] Annex XIII, Section 1.2.2 of Regulation (EU) 2017/746 - Demonstration of the analytical performance

The assay trueness has been checked by dilution test using the "WHO Reference Reagent for HEV antibody" (NIBSC Code 95/584).

A twenty-day precision study was performed according to CLSI document EP5-A3, using a coded panel of seven samples prepared by either spiking or diluting samples as necessary to obtain negative, low positive, and positive samples. The precision was determined in different manufacturer's equipment. A coded panel of seven samples was prepared by either spiking or diluting samples as necessary to obtain negative, low positive, and positive samples. Kit Controls sets were also included in the study. The panel samples and kit controls were tested with the assay in 2 replicates per run, 2 runs per day for 20 operating days on one manufacturer's equipment, on 3 assay lots. The measured CV is between 4.4% and 19.1%.

There was no carry-over/cross-contamination determined for all three validation lots tested on the different instruments of the manufacturer.

The manufacturer considered determining analytical sensitivity through seroconversion panels. Eight commercially available HEV seroconversion panels were tested using the device and two commercially available CE-marked Anti-HEV IgG comparator assays. Panels 1, 2, 3, 5, 7, and 8 obtained the same last day with non-reactive results and the first day with reactive results as in the two comparators. In Panel 4, the first days with reactive results occur seven days later than in one of the comparators. Lastly, in Panel 6, the performance (in no. of days) was more sensitive than the two comparators.

The cross-reactivity study for the anti-HEV IgG assay was designed to evaluate potential interference from antibodies to other viruses that may cause infectious diseases and other conditions. Samples for these studies were pre-screened with another commercially available hepatitis E IgG assay. If found negative for HEV IgG antibodies, those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays. The observed specificity for potentially cross-reactive specimens is comparable to open populations. No reactive results (due to potential cross-reaction) were obtained on the tested panel: anti-nuclear antibodies (ANA); auto-immune hepatitis; CMV (anti-CMV IgG and/or IgM positive); EBV (anti-EBV IgG and/or IgM positive); Fatty liver disease; Haemodialysis patients; hepatitis A virus (anti-HAV and/or HAV IgM positive); hepatitis B positive; hepatitis C virus (anti-HCV positive); hepatitis Delta (anti-HDV positive); HSV (anti-HSV IgG and/or anti-HSV IgM); HIV positive; HAMA; HTLV-1/2 (anti-HTLV positive); influenza vaccine recipients; multiparous pregnancies; multiple transfusion recipients; pregnancy 1st trimester; pregnancy 2nd trimester; and pregnancy 3rd trimester.

Controlled studies of potentially interfering substances (endogenous interferences) showed no interference of the assay to each substance listed below, at a specific concentration (triglycerides 3000 mg/dL; haemoglobin 1000 mg/dL; unconjugated bilirubin 40 mg/dL; conjugated bilirubin 40 mg/dL; cholesterol 500 mg/dL; total protein (high) 120 g/L; total protein (low) 60 g/L; and human IgG 2 g/dL). A controlled study on biotin showed no interference up to 3500 ng/mL (exogenous interferences).

Performance characteristics of cadaveric specimen testing were determined by testing postmortem specimens collected up to 24 hours after death in comparison to living donor specimens (according to the Paul Ehrlich Institute validation recommendations). The adequacy of the assay's measuring range, reported as 0.1 – 10 IU/mL, was evaluated on the results of the open HEV routine and blood donor populations, and based on the WHO Reference Reagent (NIBSC 95/584). It should be noted that International Units (IU) have not been assigned to this reference preparation which does not have the status of an WHO International Standard, and there is no metrological traceability to IU/ml. The potency of this preparation (NIBSC 95/584) is expressed in "units/ml". The confusing use of "IU/ml" applies both to further studies with results reported

with the use of this reference preparation (see below) and to the calibration of the assay and its result output in general.

The linearity of assay results is reported to range from 0.1 to 10 IU/mL HEV IgG antibodies. The methodology used does not appear to be mentioned by the manufacturer. CLSI recommends EP06-A2.

The Limit of Detection (LoD) is reported as 0.027 IU/mL. Otherwise, the Limit of Quantitation (LoQ) is 0.027 IU/mL (the highest value obtained is 0.015, but since it is lower than LoD, the LoD value will also be considered LoQ). The LoQ value obtained is consistent with the lower end of the measuring range of 0.100 IU/mL defined based on the evaluation of clinical specimens. The methodology used does not appear to be mentioned by the manufacturer. CLSI recommends EP17-A2.

All samples resulted in concentration values above the assay range expected with highly titrated sera (High-dose hook effect), indicating no sample misclassification and no high-dose saturation effect observed.

The definition of assay cut-off takes into consideration the results obtained during the clinical trials. The best cut-off positioning of the assay is in the 0.200 – 0.300 IU/mL range. The Youden's index reaches its maximum value with a cut-off of 0.239 IU/mL, but the cut-off previously defined in the feasibility phase, i.e., 0.300 IU/mL, is considered adequate to guarantee adequate specificity without affecting the sensitivity performance of the assay (diagnostic specificity 99.2%, diagnostic sensitivity 97.9%). The methodology used does not appear to be cited by the manufacturer. For example, CLSI EP24-A2.

The (metrological traceable) calibrator concentrations are referenced to the WHO Reference Reagent for Hepatitis E Antibody, NIBSC Code 95/584.

3. Expert views on the clinical performance report[3]

The diagnostic specificity was assessed on 387 expected negative specimens from healthy donors and subjects sent to a laboratory for HEV diagnosis. Specimens were screened for IgG anti-HEV antibodies with two reference CE-marked methods, and discrepant results were solved through a CE-marked immunoblot.

After testing with the device, three reactive results were observed in the expected negative population studied, leading to an overall diagnostic specificity of 99.22% (95% Confidence Interval: 97.75% - 99.74%).

The diagnostic sensitivity was assessed on 336 expected positive specimens collected in different laboratories. Specimens were screened for IgG anti-HEV antibodies with two reference CE-marked methods, and discrepant results were solved through a CE-marked immunoblot.

After testing with the device, seven non-reactive results were observed in the expected positive population studied, leading to an overall diagnostic sensitivity of 97.92% (329/336; 95% Confidence Interval: 95.76% - 98.99%). Regarding screening post-mortem specimens with the assay (claim 2), studies on both the characterization of anti-HEV IgG positive samples by HEV-NAT and, vice versa, on the characterization of HEV-NAT yields from routine blood or plasma donors using the antibody assay are missing. These studies are indispensable to recognize the virus safety benefit of the assay, if any, in connection with the screening purpose (claim 2).

We understand that the terminology "expected" should be used with caution. Samples must be from individuals diagnosed as "healthy" or “past-infection”. The term "expect" can be understood as a chance of

[3] Annex XIII, Section 1.2.3 of Regulation (EU) 2017/746 - Demonstration of the clinical performance

specimens from individuals with a false (biased) diagnosis. We also understand that more details should have been given about the individuals' diagnosis as “past-infection”.

The manufacturer states that due to the significant effect of prevalence on predictive values, they are irrelevant in retrospective studies where samples/patients were selected, and the disease prevalence is unknown. This basis is very debatable, given that several authors have published prevalences in different populations (Aslan AT, Balaban HY. Hepatitis E virus: Epidemiology, diagnosis, clinical manifestations, and treatment. World J Gastroenterol. 2020 Oct 7;26(37):5543-5560. DOI: 10.3748/wjg.v26.i37.5543. PMID: 33071523; PMCID: PMC7545399; Goel A, Vijay HJ, Katiyar H, Aggarwal R. Prevalence of hepatitis E viraemia among blood donors: a systematic review. Vox Sang. 2020 Apr;115(3):120-132. DOI: 10.1111/vox.12887. Epub 2020 Feb 6. PMID: 32030767; Chatziprodromidou IP, Dimitrakopoulou ME, Apostolou T, Katopodi T, Charalambous E, Vantarakis A. Hepatitis A and E in the Mediterranean: A systematic review. Travel Med Infect Dis. 2022 May-Jun;47:102283. DOI: 10.1016/j.tmaid.2022.102283. Epub 2022 Feb 26. PMID: 35227863; Wong RJ, Cheung R, Gish RG, Chitnis AS. Prevalence of hepatitis E infection among adults with concurrent chronic liver disease. J Viral Hepat. 2021 Nov;28(11):1643-1655. DOI: 10.1111/jvh.13597. Epub 2021 Aug 26. PMID: 34415657). We understand that the presentation of positive and negative predictive values, even if conditioned, would be necessary from the point of view of the robustness of clinical decisions based on the test results.

Once again, the methodology is not cited. CLSI suggests EP12-A3.

3.4 Views on specific assessment aspects of the performance evaluation report (PER)

(IVDR, Annex XIII, Section 1.3.2, second paragraph)

Views of the expert panel on the specific aspects included in the performance evaluation report of the manufacturer (PER)
1. The justification for the approach taken to gather the clinical evidence
The clinical performance report appears to have some limitations mainly in determining clinical sensitivity and specificity, based on the terminology used. CLSI EP12-A3 in subchapter 4.2 is clear in describing diagnostic samples. The terminology used in the PER - "expected positive specimens" and "expected negative specimens" - may lead us to assume that what was studied was the binary results' agreement and not the clinical performance. Diagnostic sensitivity and specificity require samples from diagnosed individuals. The use of an undiagnosed sample may lead to a false estimate of clinical performance. CLSI EP12-A3 (p. 41) states "If a candidate examination is evaluated by being compared with a comparative examination that is not a widely accepted best method of assessing the true condition, clinical sensitivity and clinical specificity cannot be readily estimated". Another point that does not seem well supported, as already explained, was the rationale for not determining predictive values, given that several epidemiological studies of HEV in different populations have been published.
2. The literature search methodology, protocol and report
The literature search methodology is in Section 6 of the Scientific Validity Report. In short, the manufacturer clearly articulated the topic they wanted to investigate and selected appropriate keywords and relevant databases using Boolean operators (e.g., AND, OR, NOT) to combine keywords and refine search queries. The manufacturer reviewed the search results and retrieved and organized the sources. It also evaluated the quality of the sources through exclusion and inclusion criteria. Finally, the literature was synthesized, analyzed, and adequately cited, referencing the sources used in the study. One crucial point is that the fatality rate in pregnant women is high, up to 25%. Even higher when other chronic liver diseases may be present. It should however be very clearly mentioned that these cases do occur with the genotype 1 and 2, more present in developing countries, while the genotype 3, which occurs in developed countries like the EU Member States, does not result in this dramatic loss of lives. So, pregnancy is not a risk for stillbirths or death of pregnant women in Western countries (T. P. Velavan, S.R. Pallerla, R. Johne, D. Todt, E. Steinmann, M. Schemmerer, J. Wenzel, J. Hofmann, J. Wai Kuo Shih, H. Wiedemeyer and C. Bock. Hepatitis E: An update on One Health and Clinical Medicine. Liver International 2021: 41: 1462-1473).
3. The technology on which the device is based, the intended purpose of the device and any claims made about the device's performance or safety
The manufacturer states that the assay uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of IgG antibodies to hepatitis E virus (Anti-HEV IgG) in human serum and plasma samples including specimens collected post-mortem (non-heart beating). Quantitative results are reported based on calibration of the assay with a WHO Reference Reagent (NIBSC 95/584) which has been assigned

"units/ml". Despite the current absence of a WHO International Standard (with IU assignment) for the analyte anti-HEV-IgG, the manufacturer wrongly uses the term "IU/ml" for quantitative result reporting, thus confusing metrological traceability. The assay is intended to be used as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis and to evaluate the presence and the amount of anti-HEV IgG. It is also intended to be used as a screening test for organ, tissue, and cells post-mortem donors. The assay must be performed on the manufacturer's equipment. The CLIA methodology used is recognized for its excellent performance, being used, for example, in several high-risk tests (Class D). However, the intended purpose, as already mentioned, is not consistent with the relevance of anti-HEV IgG for the diagnosis of this hepatitis or the underlying infection. Therefore, we consider that the importance of the test "as an aid in the diagnosis of HEV infection" is not consistent with the EASL Clinical Practice Guidelines on hepatitis E virus infection recommendations and its use as "a screening test for organ, tissue and cells post-mortem donors" is not supported by the current HEV diagnostic guidelines.
4. Acceptability of clinical evidence (clinical data and performance evaluation results) against state of the art in medicine
Anti-HEV IgG is typically an immunological marker of past resolved HEV infection. Due to its technical characteristics and the biological marker detected, it is this Expert Panel's view that this device should not be used to detect the presence of, or exposure to, HEV in blood, blood components, cells, tissues or organs, or in any of their derivatives, in order to assess their suitability for transfusion, transplantation or cell administration because anti-HEV IgG is considered a marker of past resolved infection characterized by absence of HEV viraemia.
5. Adequacy of PMPF report(s), where applicable
The manufacturer states in the PER "PMPF report is not available yet, since IVDR post-market processes have been implemented starting from May 26, 2022. The PMPF report and related conclusions will be referenced in the PER as soon as it will be issued for this assay."

3.5 Overall conclusions and recommendations

Overall conclusions and recommendations on the performance evaluation report
The assay based on the detection of IgG antibodies against the hepatitis E virus is described. It is accepted that only IgG antibodies do not give a conclusion of an acute infection. According to the EASL guidelines (J. Hepatology 2018 vol 68 pp1256-1271), the use of PCR technology for the detection of HEV RNA is standard care for the determination of an acute infection. A combination with IgM can be used in immunocompetent individuals, but it's mandatory in immunocompromised individuals. For HEV screening of biological materials of human origin, the detection of viral RNA as an essential component of infectious particles reflects the current state of the art. In Europe transfusion transmitted HEV infection has been documented in many countries and the most prevalent genotype is HEV genotype 3. Screening using antibody assays is often negative. (J. Izopet, P. Tremeaux, O. Marion, M. Migueres, N. Capelli, S. Chapuy-Regaud, J- M. Mansuy, F. Abravanel, N. Kamar and S. Lhomme. Hepatitis E virus infections in Europe. J. Clin. Virol. 2019.

120:20-26; H. Koot, B. Hogema, M. Koot, M. Molier and H. Zaaijer. Frequent hepatitis E in the Netherlands without traveling of immunosuppression. J. Clin. Virol. 2015, 62: 38-40).

In conclusion, the use of IgG antibodies against HEV should be used for the detection of a past infection. The marker may also be used in combination with an anti-HEV IgM detection test, only then potentially reflecting an acute infection. However, HEV RNA detection is the method of choice for the detection of an acute infection.

3.6 Stakeholder information, where available

Relevant information provided by stakeholders, if applicable[4]
Has the Secretariat provided information from stakeholders?
☐ YES ☒ NO
If yes, please summarise the information and how it was taken into account.
N/A

3.7 Divergent positions in case no consensus can be reached

In case no consensus on the views can be achieved[5], please indicate how many of the experts of the panel had divergent positions
No divergent positions.

Please summarise those divergent positions, if applicable
N/A

[4] According to Article 106.4 of Regulation (EU) 2017/745, expert panels shall take into account relevant information provided by stakeholders including patients' organisations and healthcare professionals when preparing their scientific opinions.

[5] According to Article 106.12 of Regulation (EU) 2017/745, when adopting its scientific opinion, the members of the expert panels shall use their best endeavour to reach a consensus. If consensus cannot be reached, the expert panels shall decide by a majority of their members, and the scientific opinion shall mention the divergent positions and the grounds on which they are based.