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-2023-000018
***In vitro* diagnostic medical device** *(descriptive text, no nomenclature use)*	Assay using chemiluminescent immunoassay (CLIA) technology for the qualitative determination of IgM antibodies to hepatitis E virus (Anti-HEV IgM) in human serum and plasma samples included specimens collected post-mortem (non-heart beating). It is intended as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis. It is also intended as a screening test for organ, tissue and cells post-mortem donors.

2 INFORMATION PROVIDED BY THE NOTIFIED BODY

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 IgM
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 2023-18

3.2 Summary of expert panel views

The device is an *in vitro* qualitative chemiluminescent immunoassay (CLIA) to be exclusively used on the manufacturer's platforms for the automatic qualitative determination of IgM antibodies to hepatitis E virus (anti-HEV IgM) in human serum and plasma samples (including *post-mortem* specimens). The device is intended as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis (claim 1), and as a screening test for post-mortem organ, tissue, and cells donors (claim 2).

The presence of IgM anti-HEV antibodies is considered a marker of either an active or recent infection, and a molecular test (nucleic acid test – NAT) to detect HEV RNA can be performed to confirm an active infection.

The device assay and its control set were launched under the IVDD in 2021. The supported Performance Evaluation Report (PER) document describes the characteristics and performance of the assay according to the requirements mentioned in the Performance Evaluation Report.

More precisely, the clinical performance of the device meets acceptance criteria defined in Design Input documents, showing equivalent performance compared to CE-marked kits currently available on the market, thus supporting the conclusion that the device may be considered “state of the art”.

Regarding claim 1, based on the literature review and on the different existing guidelines, the diagnosis of HEV infection in individuals with or without symptoms of hepatitis relies on the detection of HEV RNA and/or on the detection of both IgM/IgG antibodies. More precisely, the European Association for the Study of the Liver (EASL) guideline (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) and the European Centre for Disease Prevention and Control (ECDC) expert group strongly recommend using a combination of serological testing and NAT to diagnose acute HEV infection: the detection of IgM and IgG antibodies or HEV nucleic acid in serum or plasma as minimal requirement; or the detection of IgM and IgG antibodies and HEV nucleic acid in serum or plasma as optimal requirement. Accordingly, the importance of testing both anti-HEV IgM and IgG is stated in the IFU of the manufacturer, in the limitation of the procedure section. However, this diagnostic approach is less suitable for immunocompromised patients, due to a higher risk of negative results despite an infection, and for whom the NAT is requested. This statement needs to be indicated in the IFU.

For claim 2, and as stated by the manufacturer, no specific international guidelines on HEV detection in a transplantation setting have been implemented, and the cost-benefit of routine pre-transplant screening for HEV infection remains to be defined. In this context, the actual benefit of IgM screening remains to be further evaluated.

Finally, and due to its technical characteristics and to the limited information about the suitability of the marker detected, to our knowledge this type of test is currently not 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.

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 scope of the Scientific Validity Report is to demonstrate and document the association of an analyte to a clinical condition or a physiological state. The scientific validity report covers the use of anti-HEV IgM assay in human serum and plasma samples including specimens collected post-mortem as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis and as a screening test for organ, tissue and cells post-mortem donors. To develop the scientific validity report, two claims reported in the intended purpose of the device have been considered and the scientific literature has been reviewed to investigate whether the assay's claims are supported by the current scientific and clinical knowledge. Claim 1. Antibody to HEV (IgG and IgM) detection is valuable for the diagnosis of HEV infection. Claim 2. Antibody to HEV (IgG and IgM) detection is valuable for the screening of organ, tissue and cells post-mortem donors. The manufacturer has carried out a review of existing literature and available study data to collect sufficient clinical evidence to establish the use of anti-HEV IgM and anti-HEV IgG assays for the intended purposes stated above. The literature source (PubMed database) is adequate and the criteria for the literature search should cover the first and second claims for the intended purpose of both assays. The search is extensive and provides sufficient recent evidence for the scientific validity. Articles from different geographical locations are included. The literature review of the peer-reviewed scientific literature uses keywords relevant to the subject, with comparative results obtained by another device measuring the same marker. The scientific validation report conducted by the manufacturer replaces the general context of the use of biomarkers for the diagnosis and the surveillance of HEV infection. More specifically, it indicates that an acute HEV infection can be diagnosed by the detection of anti-HEV antibodies (IgM, IgG, or both) in combination with HEV RNA. Past infection is determined by the presence of anti-HEV IgG. The European Association for the Study of the Liver (EASL) recommends using a combination of serology and nucleic acid test (NAT) testing to diagnose HEV infection and only NAT testing to diagnose chronic HEV infection. The presence of IgM anti-HEV antibodies is considered a marker of either active or recent infection, and a molecular test to detect HEV RNA can be performed for confirmation. Due to the potential detection of an

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

active infection, an anti-HEV-IgM test may have some benefit in viral safety testing of biological products of human origin, obtained, e.g. from post-mortem donors.

In this context, the manufacturer simultaneously conducted the scientific validation for two devices: the device of interest, for the detection of anti-HEV IgM (and its control), but also a device for the detection of anti-HEV IgG (and its control). Thus, the conclusions provided by the manufacturer concern claims in the intended purposes of both of these devices: claim 1 with the purpose of antibody to HEV (IgG and IgM) detection for the diagnosis of HEV infections, including active infections, and claim 2 with the purpose of antibody to HEV (IgG and IgM) detection for the screening of organ, tissue, and cells post-mortem donors. This is especially true for the literature review supporting the scientific which covers the first and second claims in the intended purpose of both anti-HEV IgG and IgM assays.

Lastly, the literature review, also well detailed, appears incomplete because some references or guides/proposed standards for the diagnosis and the surveillance of HEV infection were not considered by the manufacturer in the scientific validation report, such as the 2019 ECDC technical report "Options for national testing and surveillance for hepatitis E virus in the EU/EEA" as operational guidance.

2. Expert views on the analytical performance report[2]

Analytical performance parameters have been determined based on the intended purpose of the device. The analytical performance report was conducted specifically on the device dedicated to the detection of the anti-HEV IgM, as well as on its anti-HEV IgM-associated controls. As stated by the manufacturer, the device must be performed using the manufacturer's equipment and controls anti-HEV IgM (negative and positive) are intended for use as assayed quality control samples to monitor the performance and reliability of the manufacturer's device. The performance characteristics of the device and its controls have not been established for any other assays or instrument platforms different from the manufacturer's.

The analytical report assessed the following analytical parameters:

1. Specimen types and matrices:

The choice of matrices (types of samples) is crucial to ensure the test's accuracy, sensitivity, and specificity. The most important matrices for HEV IgM testing typically include serum, plasma, whole blood, and, in some cases, lymphatic fluids. The manufacturer has tested serum and plasma (sodium citrate, sodium and lithium heparin plasma, K2-EDTA, potassium oxalate, CPDA plasma, and ACD plasma) as well as post-mortem specimens (collected up to 24 hours after death) and all of them may be used in the assay.

2. Specimen handling, collection and storage:

Specimen handling, collection, and storage are critically important when testing for anti-HEV IgM antibodies, as they can significantly impact the accuracy and reliability of test results. Proper handling and storage practices are crucial to prevent sample degradation and to ensure that the results accurately reflect the patient's immune response. The manufacturer indicates centrifugation conditions, specimen shipping conditions, storage conditions, and precautions with haemolysed or lipemic samples, microbial contamination, heat inactivation of the specimens, and air bubbles before assaying. The minimum volume required is 170 µL of specimen.

3. Accuracy of measurement:

- Trueness (bias) is not applicable since the assay has a qualitative intended use.

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

- Precision of measurement:

  The manufacturer has studied the repeatability (intra-assay precision) and the intermediate precision (inter-assay precision) through 20 × 2 × 2 precision study according to CLSI document EP5-A3, i.e. test each sample twice per run, two runs per day, for 20 or more testing days (not necessarily consecutive) using a single instrument at a single site on 3 assay lots. A panel of seven coded samples was used, each spiked or diluted as necessary to obtain negative, low positive, and positive samples, as well as kit controls. The precision of the device was evaluated for 3 different manufacturer's equipment. Depending on the equipment tested, the coefficient of variation (CV) was from 5.6% to 23.1%.

4. Carryover:

The manufacturer did not find cross-contamination for all three validation lots tested on the three analyzers. The percentage of negative results for the negative sample was 100% for both Stage A (five aliquots of a negative sample tested in two-run, two replicates) and Stage B (five aliquots of the negative sample tested in singlicate in two runs before and after a high HEV IgM positive sample). Negative samples and the percentage difference between the mean signal (RLU) values of all aliquots in Stage B and Stage A don't have an impact on clinical performance.

5. Analytical sensitivity:

Five commercially available HEV seroconversion panels were tested with the device and compared to two commercially available CE-marked anti-HEV IgM comparator assays, to determine the sensitivity of the assay, providing identical results, except for panel 3 for which the last day with non-reactive results was shorter with the device. With the background that the assay is expected to cover the early infection phase and claimed for virus safety testing of materials of human origin, the number of seroconversion panels tested appears quite low. Furthermore, data on comparative HEV-RNA detection were not presented for these seroconversion panels, preventing conclusions on the relative benefit of the assay in the early infection phase, compared to RNA detection.

6. Analytical specificity:

- Cross-reactions:

  The manufacturer performed a cross-reactivity study to evaluate potential interference from antibodies to other viruses that may cause infectious diseases, as well as from other conditions. 106 samples were pre-screened and confirmed negative with another commercial hepatitis E IgM assay, and no cross-reaction was observed in these samples with potential cross-reactants.

- Endogenous and exogenous interferences:

  No interference was observed with different classical endogenous substances, such as triglycerides, haemoglobin, unconjugated and conjugated bilirubin, cholesterol, human IgG, and proteins at a fixed concentration, as well as for biotin up to 3500 ng/mL.

- Performance on cadaveric specimens:

  No significant difference was observed when comparing the results of the device on a panel of spiked (two different levels with high negative and low positive) 20 post-mortem samples (collected up to 24 hours after death) to the same number of normal human samples from living donors, tested in parallel as reference, according to the Paul Ehrlich Institute validation protocol.

7. Measuring range of the assay:

The measuring range of the assay (i.e. 0.1 – 10 Index) was adequality evaluated on the overall tested specimens, taking into consideration the low prevalence of anti-HEV IgM in healthy donors or open HEV routine (i.e. less than 4 %). On the negative population, the manufacturer stated that the high prevalence of samples below the assay range (>60%) will not affect the assay's performance, as values are far from the cutoff and not clinically significant as specimens are always graded negative. Similarly, the prevalence of out-of-range positive specimens (>10 Index) was not considered relevant in the Technical Design Inputs, nevertheless taking into consideration that the highest result on the device is 6.92 Index, the applied assay range is adequate. Since the assay is merely qualitative, no instrumental pre-dilution is allowed. The methodology used does not appear to be mentioned by the manufacturer.

High dose hook effect: The absence of a high-dose saturation effect was observed after dilution of two high positive samples for anti-HEV IgM. Here again, the methodology used does not appear to be mentioned by the manufacturer.

8. Definition of Assay Cut-Off:

The selected cut-off of the device was fixed by the manufacturer in the range of 0.600 – 2.20 Index, based on the results obtained during the clinical trials. The Youden's index reaches its maximum value with a cutoff of 2.18 Index, but the cutoff previously defined in the feasibility phase (i.e., 1.00 Index) was considered adequate to guarantee adequate specificity without affecting the sensitivity performance of the assay (specificity 97.9%, sensitivity 98.4% on PCR positive patients. The methodology used does not appear to be cited by the manufacturer.

9. Metrological traceability:

Metrological traceability for an HEV IgM assay involves establishing a clear and documented chain of calibration that links the assay results to internationally recognized reference materials. This ensures the reliability and comparability of test results across different laboratories and testing platforms, and it is a critical aspect of assay development and quality control in clinical diagnostics, providing confidence in the accuracy of diagnostic testing for hepatitis E and other infectious diseases.

If this assay's calibration is referenced to an "in-house" preparation, the assay is calibrated using a material or standard that has been specifically prepared or developed within the laboratory where the assay is being performed, as opposed to relying on external, commercially available reference materials or certified standards. Developing and characterizing an in-house preparation requires technical expertise and resources, as well as a rigorous process of characterization and validation to ensure that it accurately represents the analyte being measured.

The linearity, detection capability (limit of blank, limit of detection, and limit of quantitation) as well as dilution linearity are not applicable since the assay has a qualitative intended use.

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

Clinical performance parameters were determined based on the intended purpose of the device. The clinical performance report was conducted specifically on the device dedicated to the detection of the anti-HEV IgM, as well as on its anti-HEV IgM-associated controls. This report provides sufficient data for demonstration of the clinical performance of the device, especially when compared with the devices of the other manufacturers.

The following performance characteristics were included in the clinical performance report:

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

1. Diagnostic specificity:

The diagnostic specificity was assessed on 484 expected negative specimens from healthy donors and subjects sent to a laboratory for routine HEV diagnosis. Specimens were screened for IgM anti-HEV antibodies with 2 reference CE-marked methods and discrepant results were solved through a CE-marked immunoblot. The overall diagnostic specificity was 97.93% (95% Confidence Interval: 96.24% – 98.87%).

2. Diagnostic sensitivity:

The diagnostic sensitivity was assessed on 156 expected positive specimens collected in different laboratories. Specimens were screened for IgM anti-HEV antibodies with 2 reference CE-marked methods and discrepant results were solved through a CE-marked immunoblot. Where available, the PCR routine method was used to confirm the HEV viremic phase.

The overall diagnostic sensitivity was 98.44% (95% CI: 91,67% - 99,72%) in the PCR-positive population studied, with 1 not-reactive result observed. The overall diagnostic sensitivity was 85,87% (95% CI: 77,31% - 91,55%) in the PCR negative or unknown population studied, with 13 reactive results observed. In this context, it appears quite unusual that there is no differentiation between PCR-negative samples and samples without PCR results, preventing more concise estimations on the potential benefit of the assay on viral safety testing of materials of human origin.

Positive and negative predictive values were not performed by the manufacturer.

Comparison with other devices on the market: A summary table comparing the diagnostic performance of 6 different manufacturers (including the device of interest), is presented, in addition to other parameters. The range of the diagnostic sensitivity is 97.1%-99.3% (with 98.44% for the device of interest), whereas the range of the diagnostic specificity is 97.6%-100% (with 97.93% for the device of interest).

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.

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 manufacturer's approach to gathering clinical evidence has addressed sufficiently the demonstration of scientific validity, analytical performance, and clinical performance. This Expert Panel is not convinced that the use of the marker anti-HEV IgM for screening of substances of human origin (claim 2) is scientifically fully justified, based on the (lack of) data presented by the manufacturer. Certain studies are still missing, and the potential benefit of these markers for screening purposes needs to be quantified, also in relation to alternative approaches, e.g. HEV-RNA screening by NAT. Furthermore, HEV does not cause a life-threatening disease with a high or suspected high risk of propagation, which is recognized in the MDCG 2020-16 rev.2 guide. Some potential benefit of anti-HEV IgM for virus safety testing of biological materials appears to be given, however, firm data on its size relative to HEV-RNA RNA detection (considered as "state of the art" virus safety test) are incomplete (see comments in "analytical sensitivity (seroconversion panels)" and "diagnostic sensitivity". For this purpose, HEV-RNA yield cases from routine screening, e.g. blood donors, would also represent another important study population. Considering the acceptable Risk-Benefit analysis, the approach taken to gather the clinical evidence was to first determine the current, generally acknowledged state of the art. An assessment of the clinical benefit of the marker and a literature review was performed and documented in the Scientific Validity Report. Then, the clinical and analytical performance claims that were established to support the IVDD-compliant product were assessed to ensure applicability to the current state of the art and compliance with the IVDR requirements. Scientific validity was based on appropriate literature search and selection. A review of existing literature and available study data was carried out to collect sufficient clinical evidence to establish the use of the anti-HEV IgM for the intended purpose of the device. Evidence of analytical performance was reported for all parameters applicable to a qualitative assay. Evidence of clinical performance was gathered from two clinical studies using clinical specimens from patients tested for HEV in human serum and plasma and by comparison to 5 existing on-market approved assays for the same analyte and intended uses. These studies also satisfy the criteria in accordance with state-of-the-art products. For the first purpose (claim 1) of the device (*i.e.* an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis), the manufacturer has provided clinical evidence, based on the analytical performance, clinical performance data, and scientific validity, to demonstrate that the device intends this purpose, but in association with anti-HEV IgG detection. The importance of testing both anti-HEV IgM and IgG is stated in the IFU of the manufacturer, in the limitation of the procedure section, as follows "The combination device IgM and IgG test and clinical data is recommended when the diagnosis of hepatitis E is based on a single specimen. A single result may not be sufficient for diagnosis but should be determined in conjunction with clinical findings, patient history and always in association with medical judgment." Besides, considering the intended purpose as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis, the expected benefits derived from the accurate information provided by

the test on the presence of Anti-HEV IgM, that facilitates interpretation, diagnosis, and related patient management decisions. It is important to realise that the presence of IgM anti-HEV antibodies is considered a marker of either active or recent infection, and a molecular test to detect HEV RNA can be performed to confirm. Clinical evidence sufficiently supports the use of this IVD for intended use.

Regarding the intended purpose as a screening test for organ, tissue, and cells post-mortem donors, the manufacturer claims that the device is valuable for the screening of organ, tissue, and cells post-mortem donors, the routine screening for antibodies against HEV, whether IgM and/or IgG is not a standard procedure for screening post-mortem donor. As stated by the manufacturer, no specific international guidelines on HEV detection in transplantation settings have been implemented, and the cost-benefit of routine pre-transplant screening for HEV infection remains to be defined. In this context, the interest in IgM screening remains to be evaluated.

Clinical performance of the device meets acceptance criteria defined in the Design Input documents as well, showing comparable performance of CE-marked kits currently available on the market, thus supporting the conclusion that the device is “state of the art”.

The manufacturer stated that considering the intended purpose as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis, the expected benefits derive from the accurate information provided by the test on the presence of anti-HEV IgM, which facilitates interpretation, diagnosis and related patient management decisions. However, no consensus or international recommendations have been established on the interpretation of anti-HEV IgM titre alone, the later one to be combined with the results of the detection of anti-HEV IgG and/ or with the detection of HEV RNA.

The manufacturer estimated that expected risks related to an erroneous result may lead to a delay in reaching the correct diagnosis while other assessments are carried out. The clinician may have to explain the incongruous result to the patient.

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 PER - "expected positive specimens" and "expected negative specimens" - may lead us to assume that it was studied the binary results’ agreement and not 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 have been published for different environments, including EU Member States.

2. The literature search methodology, protocol and report

The literature search report is developed in the Scientific Validity Report, and it is clear and extensive. An adequate literature search strategy is implemented for screening relevant publications as well as clearly defined inclusion and exclusion criteria for selecting those most consistent methodologically. The manufacturer selects appropriate keywords and relevant databases using Boolean operators (e.g., AND, OR, NOT) to combine keywords and refine search queries, focusing on English publications (guidelines, review, and Journal articles) on humans and up to 5 years old.

The databases used for this search are acceptable because they include favourable and unfavourable data, are easily searchable, and contain biomedical articles. Additionally, the databases index an adequate number of journals from different geographical locations. The time frame chosen for the initial article

search is used to obtain the most current information on recent research using current products. The manufacturer has reviewed the search results and retrieved and organized sources, as well as evaluated the quality of sources through exclusion and inclusion criteria. Finally, the manufacturer synthesizes and analyses the literature and adequately cites and references the sources used in the study.

In this context of the use of both anti-HEV IgM and IgG as biomarkers of HEV infection, the manufacturer simultaneously conducted the scientific validation for two devices: the device of interest, for the detection of anti-HEV IgM (and its control), but also a device for the detection of anti-HEV IgG (and its control). Thus, the conclusions provided by the manufacturer concern claims in the intended purposes of both of these devices: claim 1 with the purpose of antibody to HEV (IgG and IgM) detection for the diagnosis of HEV infection; and claim 2 with the purpose of antibody to HEV (IgG and IgM) detection for the screening of organ, tissue and cells post-mortem donors. This is especially true for the literature review supporting the scientific which covers the first and second claims in the intended purpose of both anti-HEV IgG and IgM assays.

For the first purpose (claim 1), the manufacturer identified 17 potentially relevant articles. Among them, 6 scientific articles were selected, from which only 2 articles were retrieved for scientific validity report, in addition to the European Association for the Study of the Liver (EASL) guidelines. The manufacturers indicated that 5 additional ones were also used, but do not appear clearly mentioned in the document. Based on this literature review, the manufacturer concluded that the detection of IgM and IgG antibodies against HEV is the first step in diagnosis. However, the presence of antibodies is not a prove of infection but allows to confirm the contact of the immune system with the virus. In all patients with anti-HEV antibodies, the evaluation of HEV-antigen and HEV-RNA is recommended. Therefore, the diagnosis of HEV requires optimally a combination of both molecular and serological techniques to confirm infection. The literature review justified the use of the device as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis. However, it does not seem appropriate to exclude an article because it is part of a book that is not freely available (e.g. Annex 1, with reference Zhao C, Wang Y. Laboratory Diagnosis of HEV Infection. Adv Exp Med Biol. 2016; 948:191-209, which was excluded for the reason “Full text is not available online or is not free for download”).

For the second purpose (claim 2), the manufacturer identified 20 potentially relevant articles, among which 12 were evaluated and only 2 were retrieved for scientific validity report (in addition to 2 other articles). Based on the literature review, as well as the opinion of the UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) and the local guidelines of the British Transplantation Society (BTS) for “Hepatitis E & Solid Organ Transplantation”, the screening of organ and tissue donor for HEV infection is recommended, using NAT for the detection of HEV viraemia. The manufacturer highlighted only one reference (Pourbaix A. et al., 2017) which suggests the systematic screening of donors by HEV RNA polymerase chain reaction and also by HEV serology, particularly in endemic regions. Thus, the second purpose of the device, based on an IgM screening test for post-mortem organ, tissue, and cells donors; is poorly supported by the literature review. However, as stated by the manufacturer, no specific international guidelines on HEV detection in transplantation settings have been implemented, and the cost-benefit of routine pre-transplant screening for HEV infection remains to be defined. In this context, the interest in IgM screening remains to be evaluated.

The literature review, also well detailed, appears incomplete because some references or guides/proposed standards for the diagnosis and the surveillance of HEV infection were not considered by the manufacturer in the scientific validation report, such as the 2019 EDCD technical report “Options for national testing and surveillance for hepatitis E virus in the EU/EEA” as operational guidance.

To date, 4 major genotypes of HEV have been identified to date, with different geographical distribution and morbidity. Although they are serologically cross-reactive, no specific studies have been performed to determine the sensitivity of the anti-HEV IgM device against these genotypes.

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 technology on which the assay is based consists of an automated chemiluminescence immunoassay (CLIA) which is a highly sensitive and specific method that relies on the detection of light emitted during a chemical reaction. Both CLIA and ELISA are immunoassay techniques that use antibodies to detect specific antigens or antibodies in a patient's blood. However, CLIA is known for its high sensitivity and automation capabilities and is particularly useful for detecting and quantifying antibodies, making it valuable for diagnosing hepatitis E infection and assessing the immune response. In conclusion, the CLIA approach is an established and recognized technology by IVD-MD stakeholders in terms of performance and safety and is adapted to the purposes of the device.

The CLIA method for detecting Hepatitis E antibodies involves using labelled antibodies (conjugates) that bind to specific immunoglobulins (IgM or IgG) in the patient's serum or plasma. When these antibodies bind to HEV antibodies in the sample, a chemiluminescent reaction occurs, producing light that can be measured and quantified. The intensity of the emitted light is directly proportional to the concentration of the target antibodies in the sample. The results of a hepatitis E CLIA test are typically reported in terms of antibody units (e.g., IU/mL) or signal-to-cutoff (S/CO) ratios, depending on the specific assay used. These results can help healthcare professionals diagnose hepatitis E infections, determine the stage of infection, and monitor a patient's immune response over time.

The intended purpose of the device is the qualitative detection of IgM antibodies to the hepatitis E virus in human serum and plasma samples including specimens collected post-mortem. The assay is intended as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis. It is also intended as a screening test for organ, tissue, and cells post-mortem donors.

The outcome of the information reported by the manufacturer supports the use of the assay in the detection of HEV IgM antibodies as an aid in HEV diagnosis. In particular, both claims according to the Scientific Validity Report are supported, i.e. the detection of HEV IgM as a useful marker for the diagnosis of HEV infection (together with IgG), and as potentially valuable for the screening of organ, tissue, and cells post-mortem donors.

In the Performance Evaluation Report, the manufacturer makes two claims. Claim 1: Antibody to HEV (IgG and IgM) detection is valuable for the diagnosis of HEV infection. Claim 2: Antibody to HEV (IgG and IgM) detection is valuable for the screening of organ, tissue, and cells post-mortem donors.

However, IgG antibodies to the hepatitis E virus are typically used for diagnosing past or resolved HEV infections and assessing immunity to the virus. They are not typically used as the primary diagnostic marker for acute HEV infection. For the diagnosis of acute HEV infection, especially during the early stages of infection, IgM antibodies to HEV are typically used. IgM antibodies appear in the bloodstream shortly after the onset of symptoms and are indicative of an active or recent HEV infection. In summary, while IgG antibodies to HEV are useful for determining past exposure and immunity to the virus, they are not the primary markers for diagnosing acute HEV infection. Anti-HEV IgM antibody is a marker of recent infection, and a positive anti-HEV IgM result with or without a positive anti-HEV IgG may confirm acute hepatitis E.

EASL strongly recommends using a combination of serological testing and NAT to diagnose an acute HEV infection. EASL lists as positive markers for an acute infection HEV antigen presence based on RNA positivity

alone or together with IgM or/and IgG positivity. If only serological testing is used, a rising IgG titre and IgM positivity are required.

The ECDC expert group suggested positivity of both IgM and IgG as the minimum criteria for the confirmation of acute HEV infection. Although a positive PCR alone (in the absence of serological test results) can be considered sufficient to confirm an acute case, PCR testing may not be available in all laboratories and countries. PCR diagnosis is considered optional in acute cases. IgM positivity may indicate a recent infection and specimens with a low level of IgM are often PCR-negative. In a minority of cases, IgM may persist for 6–12 months, while virus RNA is only detectable by PCR for 1–2 months. Regarding to ECDC expert group, the requirement for laboratory confirmation of an acute case: 1/ minimal – detection of IgM and IgG antibodies or HEV nucleic acid in serum or plasma; and 2/ optimal – detection of IgM and IgG antibodies and HEV nucleic acid in serum or plasma.

The first purpose of the device - to aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis - is under the Guidelines on Clinical Practice of Acute HEV infection by the EASL, which states that the diagnostic algorithm for the identification of acute HEV infection in immunocompetent individuals is based on the use of serology [anti-HEV IgM (and IgG)] and nucleic acid amplification testing in combination. The importance of testing both anti-HEV IgM and IgG is stated in the IFU of the manufacturer, in the limitation of the procedure section, as follows "The combination device IgM and IgG test and clinical data is recommended when the diagnosis of hepatitis E is based on a single specimen. A single result may not be sufficient for diagnosis but should be determined in conjunction with clinical findings, patient history and always in association with medical judgment."

In immunocompromised hosts, additional HEV RNA testing may be needed due to impaired immune responses and poor performance of IgM assays for this population. Only NAT testing is recommended to diagnose chronic HEV infection. This limitation is missing in the current IFU.

Regarding the justification of the second purpose of the device - the screening test for post-mortem organ, tissue, and cells donors –, the manufacturer claims that no specific international guidelines on HEV detection in a transplantation setting have been implemented and the cost-benefit of routine pre-transplant screening for HEV infection remains to be defined. However, they mentioned that the EASL recommended that blood donor services screen blood donors for HEV by NAT, informed by local risk assessment and cost-effectiveness studies. The UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) recommends HEV screening for blood components, organs, cells, and tissues and that all organ and allogeneic Haematopoietic Stem and Progenitor Cells (HSPC) donors be screened for hepatitis E. Under a universal testing strategy, SaBTO also recommends testing all tissue donors regardless of the nature of the tissue or cellular therapy. The British Transplantation Society (BTS) also developed local Guidelines for "Hepatitis E & Solid Organ Transplantation". Both SaBTO and BTS suggest screening organ and tissue donors by hepatitis E viraemia using NAT. Thus, at this stage, IgM screening for post-mortem organ, tissue, and cells donors is not supported by any of these guidelines.

Before testing cadaveric specimens, collection and centrifugation procedures should be carefully applied. After death, haemolysis and other changes (including proteolysis and dilution) occur in blood, which may lead to False Negative and False Positive in testing. In subjects transfused immediately prior to death, high percentage of haemodilution can affect the performance of the test due to analyte dilution. These limitations are indicated by the manufacturer in the IFU.

With regard to the device's safety and performance characteristics, they are based on the intended purpose of the device and are maintained throughout the product lifecycle. According to the Risk Management Report (DSI-RMR-005001) all product risks result at a low level, all known risks and any undesirable effects

were minimized leading to risk residual as low as possible. The manufacturer refers to this report in point 10 of the Performance Evaluation Report, but he does not provide that report for the purpose of being reviewed by experts.

4. Acceptability of clinical evidence (clinical data and performance evaluation results) against state of the art in medicine

The manufacturer states in Section 5 of the Performance Evaluation Report that the anti-HEV IgM device belongs to class D according to IVD-R 2017/746 in which the detection of IgM antibodies to hepatitis E virus as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis and it is also intended as a screening test for organ, tissue, and cells post-mortem donors.

Demonstration of the clinical performance of the device has been based on a combination of analytical performance data, clinical performance evaluation, and scientific peer-reviewed literature.

As indicated previously regarding the two claims of the device:

- Claim 1: Based on the literature review and on the different existing guidelines, the diagnosis of HEV infection in individuals with or without symptoms of hepatitis is based on the detection of HEV RNA and/or on the detection of both IgM/IgM antibodies. Accordingly, the importance of testing both anti-HEV IgM and IgG is stated in the IFU of the manufacturer, in the limitation of the procedure section. However, this diagnostic approach is less suitable for immunocompromised patients, due to a more important risk of false negative results, and for whom the NAT is requested. This statement needs to be indicated in the IFU.

- Claim 2: No specific international guidelines on HEV detection in transplantation settings have been implemented, and the cost-benefit of routine pre-transplant screening for active HEV infection remains to be defined, especially for an assay based on anti-HEV IgM detection. In this context, the interest in IgM screening still remains to be evaluated.

For the reasons presented, 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.

5. Adequacy of PMPF report(s), where applicable

The manufacturer has not provided a Post Market Performance Report and justifies it since IVDR post-market processes have been implemented starting from May 26, 2022. Then, the PMPF report and related conclusions will be referenced in the PER as soon as it is issued for this assay.

The manufacturer has developed a Post Market Performance Follow-up (PMPF) Plan which describes how the post-market performance follow-up activities will be carried out in compliance with the IVDR 2017/746 and Corp-GOP-000252 “Post Market Surveillance and Post Market Performance Follow-up – DSI and DSD” and Corp-GOP-000251 “Post Market Performance Follow-up (PMPF)”. This plan will address the collection and utilisation of available post-market information to allow a correct characterisation of the performance of the devices and a comparison to be made between the device and similar products available on the market.

This plan includes activities and data collection like: post-market clinical performance studies; EQAS (External Quality Assessment Scheme) designed to assess and monitor the accuracy and reliability of laboratory testing procedures and results by comparing them to external standards and reference materials, playing a crucial role in ensuring the quality of medical testing and diagnostics; commercial quality controls; perform customer satisfaction survey; review user inquires to evaluate issues in the use of the product and current scientific literature review.

3.5 Overall conclusions and recommendations

Overall conclusions and recommendations on the performance evaluation report
The information provided by the manufacturer is a comprehensive summary covering the main aspects of the Performance Evaluation Report. However, some information is lacking in the analytical performance (such as the report for the assay calibration, HEV-RNA date for seroconversion panels, etc.), and some references or guidelines appear to be missing in the scientific validity report. In the diagnostic evaluation and context with 2nd claim of the assay, a relevant study population missing so far is yield cases from routine NAT screening of blood or plasma donors. The technology is state-of-the-art, and the diagnostic specifications of the device are in-line with the other similar devices on the market. The data provided gives an overview of the analytical performance and the clinical value of the assay which is adapted for the first purpose (claim 1), acting as an aid in the diagnosis of HEV infection in immunocompetent individuals with or without symptoms of hepatitis, as the condition that both anti-HEV IgM and IgG are tested in combination (in addition or not to HEV RNA), according to the literature review and current guidelines. This diagnostic approach is less suitable for immunocompromised patients, due to a more important risk of false negative results, and for whom the NAT is requested. This statement needs to be indicated in the IFU. For the proposed second purpose (claim 2), no specific international guidelines on HEV detection in transplantation setting have been implemented, and the cost-benefit of routine pre-transplant screening for HEV infection remains to be defined for the different markers (anti-HEV IgM, HEV-RNA). In this context, the interest in IgM screening remains to be evaluated due to the potential biological limitations of this marker. HEV-RNA detection is estimated as the current “state of art” method for virus safety testing of biological products. In this context, studies on anti-HEV IgM versus HEV-RNA detection are either incomplete or missing for important specimen populations, e.g. seroconversion panels, clinical specimens, or NAT yield cases.

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

[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.

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

[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.