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Changes: §1, §9, §14, §15.1, §15.6, §15.7;
Deletions: §1, §2;

LIAISON® MUREX Anti-HEV IgM (REF 311280)

1. INTENDED PURPOSE

The LIAISON® MUREX Anti-HEV IgM assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of IgM antibodies to hepatitis E virus (Anti-HEV IgM) in human serum and plasma samples. The assay is intended as an aid in the diagnosis of HEV infection in individuals with or without symptoms of hepatitis.

The assay has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis E virus (HEV) is one of the leading causes of acute viral hepatitis with worldwide distribution and up to 20 million infections annually[1].

HEV is a small virus, with a positive-sense single-stranded ribonucleic acid (RNA) genome, identified by a single cross-reactive serotype and eight different genotypes, which characterize the geographical distribution and pathogenesis.

HEV transmission occurs through five major routes: waterborne, foodborne, zoonotic, vertical and parenteral transmission via transfusion of infected blood products[2]. The disease is common in developing countries, where it occurs mostly as outbreaks due to limited sanitation. In developed countries, HEV infection is sporadic, with foodborne transmission, due to undercooked animal meat[3]. HEV infection is usually self-limiting and resolves within 2-6 weeks, but it can also cause fulminant hepatitis, extrahepatic manifestations and a high fatality rate in pregnant women[4]. Chronic disease is common in immunosuppressed patients, such as organ transplant recipients or human immunodeficiency virus (HIV)-infected patients.

The incubation period of hepatitis E is approximately 15 to 60 days. HEV RNA is detected in blood and stool around three weeks post-infection, shortly before the onset of symptoms. Around the time of clinical onset, IgM antibodies start to appear, followed soon after by IgG antibodies. IgM antibodies are a relevant marker of acute infection and can persist for no longer than three to four months[5]. According to the EASL Clinical Practice Guidelines on hepatitis E virus infection, all patients with hepatitis should be tested for HEV, as part of the first-line virological investigation, irrespective of travel history. Moreover, a combination of both serological and molecular techniques to confirm infection in immunocompetent individuals is recommended[6]. Indeed, standard diagnostic tests for hepatitis E include the detection of HEV-specific antibodies, both anti-HEV IgM and IgG, as well as amplification of the viral genome[7]. In immunocompetent individuals, the recommendation is to test the presence of IgM anti-HEV antibodies and if the test is positive, the infection is considered either active or recent, and a molecular test to detect HEV RNA can be performed. Moreover, only if both test results (i.e., IgM anti-HEV antibodies and HEV RNA) are negative, HEV infection can be excluded. Such combination of serological and molecular tests increases the specificity and sensitivity, and thus opens the door for accurate and timely diagnosis of HEV infection[8].

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative detection of specific IgM to hepatitis E virus is an indirect chemiluminescence immunoassay (CLIA). Hepatitis E virus recombinant antigens are used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, hepatitis E virus antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with the anti-HEV IgM already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and indicates the presence or absence of IgM to hepatitis E virus in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the reagent integral.

Reagent integral

Magnetic particles (2.45 mL)	SORB	Magnetic particles (approx. ≥ 0.25%) coated with recombinant antigens (obtained in baculovirus, approx. 80 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (1.2 mL)	CAL 1	BSA, Phosphate buffer, detergents, ProClin™ 300, preservatives, an inert yellow dye.
Calibrator 2 (1.2 mL)	CAL 2	Recombinant anti-HEV IgM antibodies (approx. 1.30 Index), BSA, Phosphate buffer, detergents, ProClin™ 300, preservatives, an inert blue dye. The calibrator concentration is referenced to an 'in-house' preparation.
Specimen diluent (2 x 28 mL)	DIL SPE	BSA, TRIS buffer, detergents, ProClin™ 300, preservatives.
Conjugate (23 mL)	CONJ	Mouse monoclonal IgG antibodies to human IgM conjugated to an isoluminol derivative (minimum 10 ng/mL), non-specific mouse IgG, BSA, phosphate buffer, detergents, ProClin™ 300, preservatives, an inert blue dye.
Number of tests		100

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® Analyzer**
LIAISON® XL Cuvettes (REF X0016).	LIAISON® Module (REF 319130).
LIAISON® XL Disposable Tips (REF X0015) or	–
LIAISON® Disposable Tips (REF X0055).	–
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Starter Kit (REF 319102) or
LIAISON® EASY Starter Kit (REF 319300).	LIAISON® XL Starter Kit (REF 319200) or
–	LIAISON® EASY Starter Kit (REF 319300).
–	LIAISON® Light Check 12 (REF 319150).
LIAISON® Wash/System Liquid (REF 319100).	LIAISON® Wash/System Liquid (REF 319100).
LIAISON® XL Waste Bags (REF X0025).	LIAISON® Waste Bags (REF 450003).
–	LIAISON® Cleaning Kit (REF 310990).

LIAISON® XS Analyzer	
LIAISON® Cuvettes on Tray (REF X0053).	
LIAISON® Disposable Tips (REF X0055).	
LIAISON® EASY Starter Kit (REF 319300).	
LIAISON® EASY Wash Buffer (REF 319301).	
LIAISON® EASY System Liquid (REF 319302).	
LIAISON® EASY Waste (REF X0054).	
LIAISON® EASY Cleaning Tool (REF 310996).	

Additional required materials

LIAISON® MUREX Control Anti-HEV IgM (negative and positive) (REF 311281).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

For Laboratory Professional Use Only.

Visually inspect the integral vials for leakage at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics during the assay.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents; the waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country.

Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Pursuant to EC Regulation 1272/2008 (CLP), hazardous reagents are classified and labeled as follows:

REAGENTS:	CAL 1, CAL 2, DIL SPE, CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request. For additional information see the Safety Data Sheets available on www.diasorin.com.

7. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension. Before the seal is removed, rotate the small wheel in the magnetic particle compartment until the color of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles are resuspended. Carefully wipe the surface of each septum to remove residual liquid. Repeat as necessary until the magnetic particles are completely resuspended. Incomplete magnetic particle resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

Visually inspect the reagents, and the calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

– Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before use. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS analyzers

– LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device, which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
– Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before use. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

8. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

– **Sealed**: stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**:
  LIAISON®, LIAISON® XL and LIAISON® XS analyzers: up to **twelve** (**12**) weeks.
– Use the storage rack provided with the LIAISON® Analyzer family for upright storage of the reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate subsequent proper resuspension of the magnetic particles.
– Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

The correct type of specimens must be used with the assay. The following matrices have been tested and may be used:

– Serum;
– Plasma collected with the following anticoagulant:
• Lithium heparin;
• Sodium heparin;
• K_2-EDTA;
• Sodium citrate;
• Potassium oxalate;
• ACD (acid citrate-dextrose);
• CPDA (citrate-phosphate-dextrose-adenine).

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells, or gel separator after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.

Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.

Package and label specimens in compliance with applicable regulations covering the transportation of clinical specimens and infectious substances.

Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.

Uncontrolled transport conditions (in terms of temperature and time) may cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore, not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010).

A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells, or gel separator. The following storage conditions showed no significant differences:

– 15°-30°C for seventy-two (72) hours, in any case, room temperature storage should be avoided;
– 2°-8°C for fourteen (14) days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to 2 freeze-thaw cycles, however multiple freeze-thaw cycles should be avoided;
– Up to two (2) month at -20°C or below.

If samples are stored frozen, mix thawed samples well before testing.

Further centrifugation of specimens removed from red cells, clot, or gel separator (preferably between 3,000 and 10,000 g for 10 minutes) is recommended to guarantee the consistency of results whenever one of the following conditions is identified:

– Samples previously centrifuged and stored at 2-8°C;
– Samples with particulate matter, fibrin, turbidity, lipaemia or erythrocyte debris;
– Samples frozen and thawed;
– Samples requiring repeat testing.

Specimens with a lipid layer on the top should be transferred into a secondary tube, taking care to transfer only the clarified material.

Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Heat inactivation of the specimens may affect the test results. Check for and remove air bubbles before assaying.

The minimum volume required for a single determination is 170 µL of specimen (20 µL specimen + 150 µL dead volume).

10. CALIBRATION

Testing of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the proper cut-off.
Each calibration solution allows **five** (**5**) calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

– A new lot of reagent integral or of Starter Kit is used.
– **LIAISON®, LIAISON® XL and LIAISON® XS analyzers**: the previous calibration was performed more than **eight** (**8**) weeks before.
– Control values lie outside the expected ranges.
– **LIAISON® and LIAISON® XL analyzers**: the analyzer has been serviced.
– **LIAISON® XS Analyzer**: after a technical intervention, only if required by the service procedure, as communicated by DiaSorin Technical support or representative.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL and LIAISON® XS analyzers: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the analyzer cannot read the barcode label, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instructions.

LIAISON® XL and LIAISON® XS analyzers. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the analyzer cannot read the RFID Tag, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instructions.

The analyzer operations are as follows:

1. Dispense specimens (calibrator or control), coated magnetic particles, specimen diluent into the reaction cuvettes
2. Incubate and wash
3. Dispense the Conjugate into the reaction cuvettes
4. Incubate and wash
5. Add the Starter Reagents and measure the light emitted.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® MUREX Anti-HEV IgM controls (REF 311281).

(a) at least once per day of use,

(b) whenever the kit is calibrated,

(c) whenever a new lot of Starter Reagents is used, or in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated, and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates hepatitis E IgM antibody concentrations expressed as an Index value and grades the results. Do not dilute specimens before testing. For details, refer to the analyzer operator's manual.

Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 0.1 to 10 Index HEV IgM antibodies.

Sample results should be interpreted as follows:

LIAISON® MUREX Anti-HEV IgM assay		
Index	**Results**	**Rules and interpretation**
< 1.00	Non-Reactive	A result below 1.00 Index may indicate the absence, or a level of IgM antibodies to Hepatitis E below the threshold. The test could score non-reactive in patients during the incubation period and in the early stages of infection.
≥ 1.00	Reactive	A result above or equal to 1.00 Index generally indicates exposure of the subject to hepatitis E virus.

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® MUREX Anti-HEV IgM test is used in conjunction with other manufacturers' assays for the detection of specific Hepatitis E serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- Performance has not been established for the use of body fluids other than human.
- The combination of LIAISON® IgM and IgG test and clinical data is recommended when the diagnosis of hepatitis E is based on a single specimen. Results may remain negative during HEV infection in immunocompromised. 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.
- Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.
- Results obtained with LIAISON® MUREX Anti-HEV IgM assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Four major genotypes of HEV have been identified to date, with different geographical distribution and morbidity, but they are serologically cross-reactive. No specific studies have been performed to determine the LIAISON® MUREX Anti-HEV IgM sensitivity against genotypes.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference.
Controlled studies of potentially interfering substances showed no interference to each substance listed below in the LIAISON® MUREX Anti-HEV IgM assay, at the indicated concentration.

Substances (endogenous)	Tested concentrations
Triglycerides	3000 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Cholesterol	500 mg/dL
Human IgG	2 g/dL
Total Protein (high)	120 g/L
Total Protein (low)	60 g/L

Substances (exogenous)	Tested concentrations
Interferon alpha 2a	6000 IE/mL
Interferon alpha 2b	6000 IE/mL
Interferon alpha 1b	6000 IE/mL
Entecavir	0.5 mg/L
Tenofovir	0.0978 mg/dL
Lamivudine	300 mg/L
Adefovir dipivoxil	10 mg/L
Telbivudine	600 mg/L
Vitamin A	800 µg/dL
Vitamin B12	2850 pg/mL
Vitamin C	20 mg/dL
Vitamin D	450 ng/mL
Vitamin E	120 mg/L
Vitamin H (biotin)	3510 ng/mL
Folic Acid	160 ng/mL
Acetaminophen	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Acetylsalicylic acid	3 mg/dL
Caffeine	10.8 mg/dL
Ethanol	600 mg/dL

Cross-reactions.
The cross-reactivity study for the LIAISON® MUREX Anti-HEV IgM assay was designed to evaluate potential interference from antibodies to other viruses that may cause infectious diseases, as well as from other conditions. Samples for these studies were pre-screened with another commercially available hepatitis E IgM assay. If found negative for HEV IgM 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 that of open populations.

Condition	Number of tested samples	LIAISON® MUREX Anti-HEV IgM Reactive results
Anti-nuclear antibodies (ANA)	5	0
Auto-immune hepatitis	5	0
CMV (anti-CMV IgG and/or IgM positive)	4	0
EBV (anti-EBV IgG and/or IgM positive)	5	0
Fatty liver disease	5	0
Hemodialysis patients	5	0
Hepatitis A virus (anti-HAV IgG and IgM positive)	5	0
Hepatitis C virus (anti-HCV positive)	5	0
Hepatitis B positive	5	0
Hepatitis Delta (anti-HDV positive)	2	0
HSV (anti-HSV IgG and/or anti-HSV IgM)	5	0
HIV positive	5	0
HAMA	5	0
HTLV-1/2 (anti-HTLV positive)	5	0
Influenza vaccine recipients	5	0
Multiparous pregnancies	5	0
Multiple myeloma	5	0
Multiple transfusion recipients	5	0
Pregnancy 1st trimester	5	0
Pregnancy 2nd trimester	5	0
Pregnancy 3rd trimester	5	0
Rheumatoid factor	5	0
Total	**106**	**0**

The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

15.2. Precision with LIAISON® Analyzer

A twenty-day precision study was performed in accordance with 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. Kit Controls sets were also included in the study. The panel samples and kit controls were tested with the LIAISON® MUREX Anti-HEV IgM assay in 2 replicates per run, 2 runs per day for 20 operating days on one LIAISON® Analyzer, on 3 assay lots.

Sample ID	N	Mean Index	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
*Negative Control A	240	251	11.3	4.5%	11.5	4.6%	45.8	18.2%	0.00	0.0%	48.5	19.3%
*Negative Control B	240	252	11.1	4.4%	9.99	4.0%	46.5	18.4%	0.00	0.0%	48.8	19.4%
*Negative Control C	240	247	10.6	4.3%	9.60	3.9%	41.1	16.7%	0.00	0.0%	43.6	17.6%
Positive Control A	240	3.00	0.146	4.9%	0.124	4.1%	0.267	8.9%	0.194	6.5%	0.382	12.7%
Positive Control B	240	3.44	0.126	3.7%	0.165	4.8%	0.300	8.7%	0.258	7.5%	0.447	13.0%
Positive Control C	240	3.09	0.091	2.9%	0.150	4.9%	0.297	9.6%	0.226	7.3%	0.412	13.4%
*HEVM-01-A03	240	476	18.8	4.0%	23.8	5.0%	47.5	10.0%	21.5	4.5%	60.3	12.7%
*HEVM-01-A04	240	584	19.1	3.3%	24.8	4.3%	55.0	9.4%	10.1	1.7%	64.1	11.0%
HEVM-01-B01	240	2.01	0.066	3.3%	0.077	3.8%	0.143	7.1%	0.159	7.9%	0.237	11.8%
HEVM-01-B02	240	2.06	0.086	4.2%	0.078	3.8%	0.144	7.0%	0.142	6.9%	0.234	11.4%
HEVM-01-C01	240	2.68	0.102	3.8%	0.129	4.8%	0.203	7.6%	0.269	10.1%	0.375	14.0%
HEVM-01-D01	240	3.66	0.104	2.8%	0.165	4.5%	0.316	8.7%	0.223	6.1%	0.433	11.8%
HEVM-01-D02	240	4.87	0.148	3.0%	0.128	2.6%	0.282	5.8%	0.370	7.6%	0.505	10.4%

* Elaboration performed on RLU value since dose below the assay range.

15.3. Precision with LIAISON® XL Analyzer

A twenty-day precision study was performed in accordance with 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. Kit Controls sets were also included in the study. The panel samples and kit controls were tested with the LIAISON® MUREX Anti-HEV IgM assay in 2 replicates per run, 2 runs per day for 20 operating days on one LIAISON® XL Analyzer, on 3 assay lots.

Sample ID	N	Mean Index	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
*Negative Control A	240	458.5	61.6	13.4%	22.6	4.9%	19.0	4.1%	19.9	4.3%	71.1	15.5%
*Negative Control B	240	454.9	21.8	4.8%	12.1	2.7%	18.3	4.0%	11.4	2.5%	33.0	7.2%
*Negative Control C	240	459.5	18.6	4.0%	10.86	2.4%	19.8	4.3%	12.0	2.6%	31.6	6.9%
Positive Control A	240	2.24	0.043	1.9%	0.048	2.2%	0.079	3.5%	0.091	4.1%	0.137	6.1%
Positive Control B	240	2.28	0.041	1.8%	0.056	2.5%	0.078	3.4%	0.093	4.1%	0.140	6.1%
Positive Control C	240	2.20	0.040	1.8%	0.041	1.9%	0.076	3.4%	0.078	3.6%	0.123	5.6%
*HEVM-01-A03	240	949	54.9	5.8%	67.3	7.1%	45.9	4.8%	57.7	6.1%	114	12.0%
*HEVM-01-A04	240	1135	47.4	4.2%	31.4	2.8%	47.9	4.2%	43.3	3.8%	86.0	7.6%
HEVM-01-B01	240	1.37	0.030	2.1%	0.037	2.7%	0.047	3.4%	0.055	4.0%	0.086	6.3%
HEVM-01-B02	240	1.46	0.029	2.0%	0.051	3.5%	0.053	3.7%	0.059	4.0%	0.099	6.7%
HEVM-01-C01	240	1.86	0.034	1.8%	0.062	3.3%	0.037	2.0%	0.099	5.3%	0.127	6.9%
HEVM-01-D01	240	2.68	0.045	1.7%	0.153	5.7%	0.102	3.8%	0.081	3.0%	0.206	7.7%
HEVM-01-D02	240	3.70	0.075	2.0%	0.103	2.8%	0.070	1.9%	0.163	4.4%	0.219	5.9%

* Elaboration performed on RLU value since dose below the assay range.

15.4. Precision with LIAISON® XS Analyzer

A twelve-day precision study was performed in accordance with 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. Kit Controls sets were also included in the study. The panel samples and kit controls were tested with the LIAISON® MUREX Anti-HEV IgM assay in 2 replicates per run, 2 runs per day for 12 operating days on one LIAISON® XS Analyzer, on 3 assay lots.

Sample ID	N	Mean Index	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
*Negative Control A	144	319	22.4	7.0%	70.2	22.0%	2.00	0.6%	1.97	0.6%	73.7	23.1%
*Negative Control B	144	317	18.2	5.7%	62.6	19.7%	0.00	0.0%	9.92	3.1%	65.9	20.8%
*Negative Control C	144	319	14.0	4.4%	54.3	17.0%	0.00	0.0%	12.70	4.0%	57.4	18.0%
Positive Control A	144	2.54	0.145	5.7%	0.110	4.3%	0.185	7.3%	0.081	3.2%	0.272	10.7%
Positive Control B	144	2.56	0.114	4.5%	0.134	5.3%	0.193	7.6%	0.109	4.3%	0.284	11.1%
Positive Control C	144	2.51	0.125	5.0%	0.077	3.1%	0.212	8.5%	0.110	4.4%	0.280	11.2%
*HEVM-01-A03	144	861	31.4	3.6%	33.8	3.9%	137	16.0%	65.5	7.6%	159	18.5%
*HEVM-01-A04	144	1003	28.7	2.9%	28.5	2.8%	108	10.8%	52.6	5.2%	127	12.6%
HEVM-01-B01	144	1.45	0.105	7.3%	0.076	5.2%	0.071	4.9%	0.085	5.9%	0.170	11.8%
HEVM-01-B02	144	1.49	0.100	6.7%	0.086	5.8%	0.108	7.3%	0.136	9.1%	0.218	14.6%
HEVM-01-C01	144	1.99	0.110	5.6%	0.062	3.1%	0.123	6.2%	0.183	9.2%	0.254	12.8%
HEVM-01-D01	144	2.72	0.125	4.6%	0.151	5.6%	0.169	6.2%	0.148	5.5%	0.298	11.0%
HEVM-01-D02	144	4.03	0.305	7.6%	0.205	5.1%	0.250	6.2%	0.256	6.4%	0.513	12.7%

* Elaboration performed on RLU value since dose below the assay range.

15.5. Analytical Sensitivity as Seroconversion Panel Performance

Five (5) commercially available HEV seroconversion panels were tested using LIAISON® MUREX Anti-HEV IgM and two commercially available CE-marked Anti-HEV IgM comparator assays, to determine the sensitivity of the assay. The results are summarized in the following table:

Panel ID	LIAISON® MUREX Anti-HEV IgM		Anti-HEV IgM Comparator assay (A)		Anti-HEV IgM Comparator assay (B)	
	Last day with non-reactive results	First day with reactive results	Last day with non-reactive results	First day with reactive results	Last day with non-reactive results	First day with reactive results
Panel 1	38	41	38	41	38	41
Panel 2	25	35	25	35	25	35
Panel 3	25	39	114	n.a.	114	n.a.
Panel 4	21	28	21	28	21	28
Panel 5	46	50	46	50	46	50

15.6. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results because the analytical signals remain consistently high (saturation curve).

High dose saturation effect was evaluated by diluting two high positive samples for hepatitis E IgM and no high-dose saturation effect was observed.

15.7. Diagnostic specificity and sensitivity

Diagnostic specificity.

The diagnostic specificity was assessed on four hundred and eighty-four (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.

Results are reported in the Table below:

Populations	Number of cases	LIAISON® MUREX Anti-HEV IgM	Diagnostic Specificity %	Diagnostic Specificity 95% C.I.
Healthy Donors	199	194/199	97.49%	94.25% – 98.92%
From HEV diagnostic routine	285	280/285	98.25%	95.96% – 99.25%
Overall	484	474/484	97.93%	96.24% – 98.87%

Diagnostic sensitivity.

The diagnostic sensitivity was assessed on one hundred and fifty-six (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.

Results are shown in the Table below:

Populations	Number of cases	LIAISON® MUREX Anti-HEV IgM	Diagnostic Sensitivity %	Diagnostic Sensitivity 95% C.I.
PCR positive	64	63/64	98.44%	91.67% – 99.72%
PCR negative	52	50/52	96.15%	87.02% – 98.94%
PCR unknown	40	29/40	72.50 %	57.17% - 83.89%

The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Combined anti-HEV IgG and IgM results

Considering LIAISON® assays and reference methods results on 715 samples, the following frequency rates were obtained in the study, for IgG and IgM combined results:

Anti-HEV IgG	Anti-HEV IgM	Interpretation of results	LIAISON® assays	Reference CE marked assays 1	Reference CE marked assays 2
Positive	Positive	acute HEV infection	73 10.2%	53 7.4%	62 8.7%
Negative	Positive	early HEV infection	5 0.7%	6 0.8%	9 1.3%
Positive	Negative	past HEV infection	241 33.7%	267 33.7%	257 35.9%
Negative	Negative	No HEV infection	396 55.4%	389 54.4%	387 54.1%

Summary of safety and performance is available on EUDAMED.

For EU only: please be aware that any serious incident that has occurred in relation to this IVD medical device should be reported to DiaSorin Italia S.p.A. and to the Competent Authority of the EU Member State in which the user and/or the patient is established.

REFERENCES

1. WHO. Hepatitis E: Fact Sheet. 2020. Available online: https://www.who.int/news- room/fact-sheets/detail/hepatitis-e (accessed on 01 March 2021);
2. Khuroo MS. et al. “Hepatitis E: an emerging global disease - from discovery towards control and cure.” J Viral Hepat. 2016 Feb; 23(2):68-79;
3. Kamar N. et al. “Hepatitis E virus infection.” Nat Rev Dis Primers. 2017 Nov; 16 (3):17086;
4. Lhomme S. et al. “Clinical Manifestations, Pathogenesis and Treatment of Hepatitis E Virus Infections.” Journal of clinical medicine. 2020 Jan; 9(2):331;
5. Al-Sadeq DW. et al. “Laboratory challenges in the diagnosis of hepatitis E virus.” J Med Microbiol. 2018 Apr; 67(4):466-480;
6. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol. 2018 Jun; 68(6):1256-1271;
7. Pallerla SR. et al. “Hepatitis E Virus Infection: Circulation, Molecular Epidemiology, and Impact on Global Health” Pathogens. 2020, 9 (856);
8. Talapko, J. et al. “Towards the Improved Accuracy of Hepatitis E Diagnosis in Vulnerable and Target Groups: A Global Perspective on the Current State of Knowledge and the Implications for Practice.” Healthcare. 2021, 9 (133);
9. Pourbaix A. et al. “Evidence of hepatitis E virus transmission by renal graft.” Transpl Infect Dis. 2017 Feb; 19(1).

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