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Changes: §4, §6, §15.1, §16, References;
Deletions: -

LIAISON® XL murex Anti-HDV (REF 311260)

1. INTENDED PURPOSE

The LIAISON® XL MUREX Anti-HDV assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of antibodies to hepatitis D virus (anti-HDV) in human serum and plasma samples included specimens collected post-mortem (non-heart beating).
The assay is intended as an aid in the diagnosis of HDV 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 assay has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis delta virus (HDV), identified in 1977, is the smallest human RNA virus with a circular RNA genome of approximately 1,700 bases; the genome is single-stranded negative sense and forms a covalently closed circle [1,2]. The RNA encodes a protein called the delta antigen, which is subsequently encased in an envelope embedded with the hepatitis B surface antigen (HBsAg). HDV infection is significant because, although it suppresses hepatitis B virus (HBV) replication, it can cause severe liver disease that may include fulminant liver failure and rapid progression to cirrhosis and hepatic decompensation, as well as an increased risk of liver cancer [3].
Since HDV can only cause infection in the presence of HBV, it was thought that the widespread introduction of HBV vaccine would ultimately result in a decreased prevalence of HDV. In addition, a decrease in the prevalence of HDV was observed during the second decade after its discovery. This led to decreased awareness and testing for HDV, which further contributed to the perception that the virus was on its way to being eradicated. Unfortunately, recent studies have shown the contrary and, currently, high prevalence remains in many parts of the world [4,5,6].
Worldwide, it is estimated that 15–20 million people are HDV infected, with a widely varying prevalence, depending on the region. The highest prevalence is seen in the Mediterranean basin, the Middle East, central and northern Asia, western and central Africa, the Amazonian basin, the Pacific islands and Vietnam. HDV antibodies were found in up to 30% of chronic hepatitis B (CHB) patients in some countries [7,8].
At least eight genotypes of HDV have been identified; genotype 1 is most common throughout the world, genotype 2 is seen in the Far East, and genotype 3 is present in the northern part of South America. Genotypes 5–8 have been identified in African patients, and genotype 4 is found in Taiwan and Japan [9,11].
The way of transmission of HDV is similar to that of HBV: percutaneous exposure (injecting drug users), permucosal, sexual contact and perinatal (rare). Blood is potentially infectious during all phases of active hepatitis D infection. Peak infectivity probably occurs just before the onset of acute disease. There is a 5% risk of fulminant hepatitis in co-infection. Otherwise, the prognosis of co-infection is generally good, whereas the prognosis for superinfection is variable [10].
The development of anti-HDV antibodies, either of IgM or IgG subtype, is universal in individuals with HDV infection and is an expression of the innate and adaptive response by the infected host.
All HBsAg-positive patients should be tested for anti-HDV antibodies. IgG anti-HDV persists for several years even after the clearance of HDV infection. By contrast, IgM anti-HDV declines in patients with self-limited infections, but persists over time in patients whose infection progresses to chronicity. HDV infection should be then confirmed by the detection of serum HDV RNA. As HDV is dependent on HBV and assessing HBV markers and replication is necessary in order to establish the diagnosis and liver disease, HBsAg levels seem to correlate with HDV viremia in chronic HDV carriers [12].
As many hepatotrophic viruses, also Hepatitis Delta virus (HDV) affect solid organ donors and recipients. Therefore, the diagnosis of virus infections in the transplant setting is important in order to optimize the selection of organ donors and the management of viral hepatitis in post-transplant patients.
Different national Transplant Societies highlight the need to evaluate HDV infection on liver transplantation in particular.
Guidelines from the British Transplant Society (BTS) on the management of hepatitis B in the transplant setting admit HBsAg positive donation for any liver recipient. Otherwise, If the donor results HBV/HDV co-infected, the liver should not be used [13].

3. PRINCIPLE OF THE PROCEDURE

The method for the qualitative determination of specific antibodies to hepatitis D virus (HDV) is an indirect chemiluminescence immunoassay (CLIA). The recombinant antigen specific for HDV is used for coating magnetic particles (solid phase). During the first incubation, the HDV antibodies present in the calibrator, samples or controls bind to the solid phase through the recombinant HDV antigen. During the second incubation, mouse monoclonal antibodies to human IgG and mouse monoclonal antibodies to human IgM, both linked to an isoluminol derivative (isoluminol-antibody conjugate), react with antibodies to HDV 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 is indicative of antibodies to HDV presence in the calibrator, samples or controls.

* (LIAISON®, LIAISON® XL)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with biotinylated recombinant HDAg (obtained in E. coli) (minimum 15 µg/mL), streptavidin, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (0.7 mL)	CAL 1	BSA, phosphate buffer, EDTA, 0.2% ProClin™ 300, an inert yellow dye. The calibrators concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (0.7 mL)	CAL 2	Diluted and inactivated serum/plasma containing low anti-HDV levels (approx. 1 AU/mL), BSA, phosphate buffer, EDTA, 0.2% ProClin™ 300, an inert blue dye. The calibrators concentrations (AU/mL) are referenced to an in-house antibody preparation.
Specimen diluent (25 mL)	DIL SPE	BSA, casein, borate buffer, EDTA, 0.2% ProClin™ 300, detergents and preservative.
Conjugate (23 mL)	CONJ	Mouse monoclonal IgG to human IgG and Mouse monoclonal IgG to human IgM (minimum 10 ng/mL) conjugated to an isoluminol derivative, aspecific mouse IgG, BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives, an inert blue dye.
Number of tests		100

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

Materials required but not provided

LIAISON® XL Analyzer	LIAISON® Analyzer
LIAISON® XL Cuvettes (REF X0016).	LIAISON® Module (REF 319130).
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® Starter Kit (REF 319102) or
LIAISON® Disposable Tips (REF X0055).	LIAISON® XL Starter Kit (REF 319200) or
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® EASY Starter Kit (REF 319300).
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).

Additionally required materials

LIAISON® XL MUREX Anti-HDV controls (Negative and Positive (REF 311261)).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use. For Laboratory Professional Use Only.
Visually inspect the integral vials for leaking at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately
All human serum and plasma units used to produce the calibrator 2 provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive for anti-HCV, anti-HIV-1/2 and reactive for HBsAg together with HDV antibodies. The anti-HDV positive units have been inactivated by chemical and heat treatment, however they may derive from HDV-infected patients and, therefore, should be considered as potentially infectious.
Since no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
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.
The LIAISON® and LIAISON® XL analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.
Do not use kits or components beyond the expiration date given on the label.

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

REAGENTS:	CAL 1, CAL 2, CONJ	DIL SPE
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 Aquatic chronic 3 H412 Repr 1B H360FD
SIGNAL WORD:	Warning	Danger
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	 GHS07 Exclamation mark  GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. H360FD May damage fertility. May damage the unborn child
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.	P261 Avoid breathing dust/fume/gas/mist/ vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P363 Wash contaminated clothing before reuse. P202 Do not handle until all safety precautions have been red and understood. P308+P313 If exposed or concerned: get medical advice/attention.
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).	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). Boric acid.

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. REAGENT PREPARATION

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 at the magnetic particle compartment until the colour 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 have been resuspended. Carefully wipe the surface of each septum to remove residual liquid.
Repeat as necessary until the magnetic particles are completely resuspended.
An 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, the calibrators in particular (position two and three following the magnetic particle vial), to ensure that no foaming is 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 using. 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 Analyzer

– LIAISON® XL Analyzer is 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.

CONTROLS
Refer to the LIAISON® XL MUREX Anti-HDV Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY
– **Sealed**: Stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**: Stability ten (10) weeks.
– Always use the same analyzer type for an already opened reagent integral.
– Use the storage rack provided with the analyzer for upright storage of the reagent integral.
– Do not freeze.
– Keep upright for storage to later facilitate the proper resuspension of magnetic particles.
– Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION
The correct specimen type must be used in the assay. Following matrices have been tested and may be used:
– serum,
– lithium and sodium heparin plasma,
– K2-EDTA plasma,
– sodium citrate plasma,
– potassium oxalate plasma.

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.
Post-mortem specimens, collected up to 24 hours after death, have been tested and may be also used in the assay.
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 transport of clinical specimens and infectious substances.
Specimens may be shipped in dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.
Uncontrolled transport and storage 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:
– room temperature storage should be avoided;
– 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to 6 freeze-thaw cycles, however multiple freeze thaw cycles should be avoided.

If samples are stored frozen, mix thawed samples well before testing.
Further centrifugation of specimens removed from red cells, clot or gel separator (suggested 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.
Cadaveric specimens should be stored following same indications than for living donors.
The minimum volume required for a single determination is 170 µL specimen (20 µL specimen + 150 µL dead volume).

10. CALIBRATION
Testing assay specific calibrators allows the detected relative light unit (RLU) values to adjust the proper cut-off. Each calibrator solution allows **four (4)** calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
– A new lot of Starter Kit is used.
– The previous calibration was performed more than **six (6)** weeks before.
– Each time a new lot of integral is used.
– Control values lie outside the expected ranges.
– **LIAISON® and LIAISON® XL analyzers:** the analyzer has been serviced.
– LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
– LIAISON® XL Analyzer: 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 bar code label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instructions.

LIAISON® XL Analyzer. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, 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 specimen diluent into the cuvettes / reaction module.
2. Dispense coated magnetic particles.
3. Dispense calibrators, controls or specimens.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugates into the cuvettes / reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Reagents and measure the light emitted.

12. QUALITY CONTROL

LIAISON® XL controls should be run in singlicate to monitor assay performance. Quality control must be performed by running LIAISON® XL MUREX Control Anti-HDV (REF 311261)
(a) at least once per day of use, before running the test,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
(e) in agreement with guidelines or the 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 the controls retested. If the control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If the control values lie outside the expected ranges, the 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 the quality control materials used.

13. INTERPRETATION OF RESULTS

The presence or absence of HDV antibodies in the specimens is qualitatively determined by comparing the chemiluminescence reaction signal to the specific assay calibration. The analyzer automatically calculates the AU/mL (Arbitrary Units per milliliter) value and grades the results. For details, refer to the analyzer operator's manual.
Calibrators and controls may give different RLU or dose results on LIAISON® and LIAISON® XL, but patient results are equivalent.

Assay range

0.100 to 30.0 AU/mL value.
The test is qualitative, thus predilution of over-range specimens at first test, is not considered.
The cut-off value discriminating between the presence and the absence of HDV antibodies is 1.00 AU/mL.
Specimens with AU/mL below 1.00 are considered non-reactive for HDV antibodies.
Specimens with AU/mL above or equal to 1.00 are considered reactive for HDV antibodies.
A reactive specimen should be investigated further with sensitive, supplemental HDV-specific tests, such as identification of HDV RNA, to be matched with the clinical story of the subject and with markers of a previous HBV infection (HBsAg, anti-HB Core, HBV DNA, etc.).
Like all immunoassays, the LIAISON® XL MUREX Anti-HDV assay may occasionally yield non-specific reactions due to other causes.
A non-reactive test result for HDV antibodies does not exclude the possibility of exposure to or infection with HDV. Further investigations with alternative HDV specific tests are suggested in case of suspected infection despite the negative finding.
If LIAISON® XL MUREX Anti-HDV results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

14. LIMITATIONS OF THE PROCEDURE

WARNING: This test is suitable only for investigating individual samples, not for diluted specimens, sample pools or heat-inactivated specimens.

- Assay performance characteristics have not been established when any LIAISON® XL MUREX Anti-HDV test is used in conjunction with other manufacturers' assays for detection of specific HDV 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.
- Falsely reactive results cannot be ruled out with any test kit, the percentage of which is related to specimen integrity, the specificity of the test kit, and the prevalence of the anti-HDV antibodies in the population being screened.
  However, diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures, as well as in association with medical judgment.
- Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may interfere in a monoclonal antibody-based immunoassay and their results should be evaluated with care (for details, refer to 15.1 Analytical specificity page 6).
- Specimens from patients receiving therapeutic doses of Biotin (vitamin H, B_7 or B_8) may interfere in immunoassays based on biotinylated reagents. No interference was observed testing Biotin serum concentration of 3500 ng/mL with LIAISON® XL MUREX Anti-HDV assay.
- Before testing cadaveric specimens, proper collection and centrifugation procedures should be applied. After death, haemolysis and other changes (including proteolysis and dilution) occur in blood, which may lead to False Negative or False Positive results in testing. In subjects transfused immediately prior to death a high percentage of haemodilution can affect the performance of the test due to analyte.
- Results obtained with LIAISON® XL MUREX Anti-HDV assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.

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 or conditions showed that the assay performance was neither affected by anticoagulants (sodium citrate, K2 EDTA, lithium and sodium heparin, potassium oxalate), nor by the following compounds.

Tested Compound	Tested concentration
Unconjugated bilirubin	20 mg/dL
Conjugated bilirubin	20 mg/dL
Haemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Hypergammaglobulin (Immunoglobulin G)	60 g/L
Total protein (high)	120 g/L
Total protein (low)	<60 g/L

In addition, controlled studies of potentially exogenous substances showed no interference to each substance listed below in the LIAISON® XL MUREX Anti-HDV, at the indicated concentration

Tested Compound	Tested concentration
Biotin	3500 ng/mL
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

Cross-reactions

The cross-reactivity study for the LIAISON® XL MUREX Anti-HDV assay was designed to evaluate potential interference from antibodies to other organisms that may cause infectious diseases (Saccharomyces, Borrelia burgdorferi, Chlamydia trachomatis, hCMV, EBV, HAV, HBV, HCV, HIV, HSV, HTLV-I/II, *Neisseria gonorrhea, Measles, Mumps, Mycoplasma Pneumoniae*, Parvovirus B19, Rubella virus, *Toxoplasma gondii, Treponema pallidum, Trypanosoma cruzi*, VZV), from diseases that involve the liver (liver cancer, fatty liver disease), as well as from other conditions that may involve immune system activity (anti-nuclear autoantibodies, autoimmune hepatitis, rheumatoid factor, human anti-mouse antibodies, monoclonal gammopathies, influenza vaccine, multiple myeloma, anti-*E. coli* antibodies, multiple transfusion recipients, hemodialysis patients, pregnancies). Samples for these studies were pre-screened with another commercially available anti-HDV assay. If found negative for HDV 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. Specificity observed in potentially cross-reactive specimens is comparable to that of open populations.

Condition	Number of expected negative samples	LIAISON® XL MUREX Anti-HDV positive results
Anti-Saccharomyces antibodies	5	0
Borrelia burgdorferi antibodies	6	0
Chlamydia trachomatis antibodies	4	0
hCMV antibodies	17	0
EBV (VCA) antibodies	5	0
HAV antibodies	5	0
HBV antibodies	7	0
HCV antibodies	5	0
HIV I antibodies	5	0
HIV I-O antibodies	2	0
HIV II antibodies	6	0
HSV antibodies	16	0
HTLV-I/II antibodies	4	0
Neisseria gonorrhea antibodies	4	0
Measles virus antibodies	4	0
Mumps virus antibodies	4	0
Mycoplasma pneumoniae antibodies	3	0
Parvovirus B19 antibodies	4	0
Rubella virus antibodies	13	0
Toxoplasma gondii antibodies	10	0
Treponema pallidum antibodies	6	0
Trypanosoma cruzi antibodies	5	0
VZV antibodies	12	0
Anti-nuclear autoantibodies (ANA)	4	0
Human anti-mouse antibodies (HAMA)	15	0
Rheumatoid factor (anti-Fc immunoglobulin)	21	0
Autoimmune hepatitis	8	0
Fatty liver disease	4	0
Liver cancer (Hepatocellular carcinoma)	5	0
Monoclonal Gammopathy IgM	3	0
Monoclonal Gammopathy IgG	4	0
Vaccinated against Influenza	5	0
Multiple Myeloma	5	0
Multiple Transfusion Recipients	10	1
Anti-E.coli antibodies	5	0
Hemodialysis patients	11	0
Total	252	1

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). **The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations**.

Repeatability

Twenty replicates on four samples were performed in the same run to evaluate in-house repeatability. The obtained percent coefficient of variation (CV%) did not exceed 10%.

Repeatability	A	B	C	D
Number of determinations	20	20	20	20
Means (AU/mL)	1.00	1.40	2.82	2.04
Standard deviation (AU/mL)	0.052	0.039	0.109	0.115
Coefficient of variation (%)	**5.2%**	**2.8%**	**3.9%**	**5.6%**
Min. value (AU/mL)	0.88	1.30	2.52	1.69
Max. value (AU/mL)	1.06	1.46	2.93	2.11

Reproducibility

Twenty replicates on four samples were performed on different days (one or two runs per day) with three different lots of integrals to evaluate reproducibility. The tests were performed in two sites, in-house (site 1) and in an independent laboratory (site 2), with one instrument per site. The obtained percent coefficient of variation (CV%) did not exceed 15%.

Reproducibility - Site 1	**A**	**B**	**C**	**D**
LOT No. 01				
Number of determinations	20	20	20	20
Means (AU/mL)	1.06	1.40	2.90	2.20
Standard deviation (AU/mL)	0.04	0.10	0.13	0.08
Coefficient of variation (%)	**4.2%**	**5.0%**	**4.5%**	**3.8%**
Min. value (AU/mL)	0.994	1.30	2.6	2.00
Max. value (AU/mL)	1.15	1.50	3.1	2.30
LOT No. 02				
Number of determinations	20	20	20	20
Means (AU/mL)	1.00	1.40	2.90	1.90
Standard deviation (AU/mL)	0.06	0.10	0.19	0.12
Coefficient of variation (%)	**5.9%**	**7.4%**	**6.7%**	**6.5%**
Min. value (AU/mL)	0.856	1.10	2.30	1.70
Max. value (AU/mL)	1.10	1.50	3.10	2.20
LOT No. 03				
Number of determinations	20	20	20	20
Means (AU/mL)	1.00	1.40	2.90	2.20
Standard deviation (AU/mL)	0.08	0.12	0.23	0.09
Coefficient of variation (%)	**7.9%**	**8.4%**	**7.9%**	**4.2**%
Min. value (AU/mL)	0.860	1.20	2.50	2.00
Max. value (AU/mL)	1.14	1.60	3.30	2.40
Inter-lot coefficient of variation (%)	6.6%	7.0%	6.5%	5.1%

Reproducibility - Site 2	**A**	**B**	**C**	**D**
LOT No. 01				
Number of determinations	20	20	20	20
Means (AU/mL)	1.09	1.44	3.00	2.29
Standard deviation (AU/mL)	0.07	0.11	0.32	0.17
Coefficient of variation (%)	**6.7%**	**7.7%**	**10.6%**	**7.3%**
Min. value (AU/mL)	0.920	1.12	1.86	1.82
Max. value (AU/mL)	1.21	1.63	3.42	2.53
LOT No. 02				
Number of determinations	20	20	20	20
Means (AU/mL)	1.16	1.66	3.30	2.40
Standard deviation (AU/mL)	0.04	0.09	0.18	0.21
Coefficient of variation (%)	**3.8%**	**5.3%**	**5.5%**	**8.9%**
Min. value (AU/mL)	1.09	1.54	2.73	2.40
Max. value (AU/mL)	1.25	1.92	3.69	2.72
LOT No. 03				
Number of determinations	20	20	20	20
Means (AU/mL)	1.12	1.59	3.36	2.46
Standard deviation (AU/mL)	0.09	0.15	0.24	0.25
Coefficient of variation (%)	**8.3%**	**9.7%**	**7.1%**	**10.3%**
Min. value (AU/mL)	0.810	1.14	2.69	2.05
Max. value (AU/mL)	1.24	1.79	3.71	2.89
Inter-lot coefficient of variation (%)	6.9%	9.6%	9.2%	9.3%

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). **The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.**

Repeatability

Twenty replicates on four samples were performed in the same run to evaluate in-house repeatability. The obtained percent coefficient of variation (CV%) did not exceed 10%.

Repeatability	A	B	C	D
Number of determinations	20	20	20	20
Means (AU/mL)	1.15	1.72	3.39	2.37
Standard deviation (AU/mL)	0.026	0.026	0.043	0.003
Coefficient of variation (%)	**2.2%**	**1.5%**	**1.3%**	**1.4%**
Min. value (AU/mL)	1.12	1.68	3.28	2.29
Max. value (AU/mL)	1.21	1.77	3.45	2.42

Reproducibility

Twenty replicates on four samples were performed on different days (one or two runs per day) with three different lots of integral to evaluate reproducibility. The tests were performed in two sites, in-house (site 1) and in an independent laboratory (site 2), with one instrument per site. The obtained percent coefficient of variation (CV%) did not exceed 15%.

Reproducibility - Site 1	A	B	C	D
LOT No. 01				
Number of determinations	20	20	20	20
Means (AU/mL)	1.12	1.50	2.90	2.40
Standard deviation (AU/mL)	0.103	0.194	0.418	0.161
Coefficient of variation (%)	**9.2%**	**13.0%**	**14.4%**	**6.7%**
Min. value (AU/mL)	0.839	1.10	2.10	2.10
Max. value (AU/mL)	1.25	1.70	3.50	2.60
LOT No. 02				
Number of determinations	20	20	20	20
Means (AU/mL)	1.10	1.70	3.30	2.40
Standard deviation (AU/mL)	0.118	0.152	0.412	0.324
Coefficient of variation (%)	**10.8%**	**8.9%**	**12.5%**	**13.5%**
Min. value (AU/mL)	0.833	1.40	2.30	1.60
Max. value (AU/mL)	1.27	1.80	3.70	2.70
LOT No. 03				
Number of determinations	20	20	20	20
Means (AU/mL)	1.17	1.74	3.46	2.42
Standard deviation (AU/mL)	0.137	0.141	0.493	0.338
Coefficient of variation (%)	**11.8%**	**8.1%**	**14.3%**	**13.9%**
Min. value (AU/mL)	0.890	1.48	2.55	1.67
Max. value (AU/mL)	1.35	1.94	4.15	2.95
Inter-lot coefficient of variation (%)	10.8%	12.3%	15.2%	11.8%

Reproducibility - Site 2	A	B	C	D
LOT No. 01				
Number of determinations	20	20	20	20
Means (AU/mL)	1.12	1.60	3.25	2.61
Standard deviation (AU/mL)	0.111	0.153	0.25	0.240
Coefficient of variation (%)	**9.9%**	**9.5%**	**7.8%**	**9.2%**
Min. value (AU/mL)	0.900	1.39	2.81	2.22
Max. value (AU/mL)	1.35	1.88	3.98	3.22
LOT No. 02				
Number of determinations	20	20	20	20
Means (AU/mL)	0.97	1.36	2.83	2.14
Standard deviation (AU/mL)	0.091	0.170	0.239	0.244
Coefficient of variation (%)	**9.4%**	**12.5%**	**8.4%**	**11.4%**
Min. value (AU/mL)	0.830	1.01	2.46	1.71
Max. value (AU/mL)	1.11	1.76	3.26	2.57
LOT No. 03				
Number of determinations	20	20	20	20
Means (AU/mL)	1.20	1.77	3.53	2.70
Standard deviation (AU/mL)	0.098	0.195	0.470	0.207
Coefficient of variation (%)	**8.2%**	**11.0%**	**13.3%**	**7.7%**
Min. value (AU/mL)	1.03	1.46	2.24	2.38
Max. value (AU/mL)	1.39	2.17	4.43	3.17
Inter-lot coefficient of variation (%)	12.6%	15.3%	13.7%	13.5%

15.4. High-dose saturation effect

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

15.5. Performance characteristics of cadaveric specimen testing

Performance characteristics of cadaveric specimen testing were determined by testing, according to the PEI validation protocol*, post-mortem specimens collected up to 24 hours after death in comparison to living donor specimens. 20 post-mortem samples were tested as unspiked and spiked at 2 levels: low positive and medium/high positive. The same procedure was performed with the same number of normal human serum from living donors, tested in parallel as reference to compare with post-mortem sample results. The results obtained were analyzed through the calculation of the percentage difference between the mean of living donor results and the mean of post-mortem results, at each reactivity level. In this study, the obtained percentage difference was equal to or below 4.4% for each of the tested reactivity levels (see table below). Paired t-test analysis was performed between post-mortem and living donor specimens, spiked at low and medium/high positive levels, demonstrating no significant difference between the two groups (p value < 0.05).
Repeatability was assessed using one post-mortem and one living donor specimen, spiked up to a low-level of reactivity with a human serum reactive for antibodies to hepatitis D virus (HDV). Each specimen was assessed in six replicates in the same run. The obtained percent coefficient of variation (CV%) did not exceed 15%. As reported in the table below, 1.3% for the cadaveric specimen and 1.1% for the living donor were found in the study. **The results refer to the group of investigated samples and are not guaranteed specifications, as differences may exist between laboratories and locations.**

	Sample	Test Results Means (AU/mL)	Recovery (%) post-mortem / living donors	t-test p value	CV% 6 replicates
Neat	Post-Mortem unspiked	< 0.100	n.a.	n.a.	n.a.
	Living Donor unspiked	< 0.100			
Low Positive	Post-Mortem spiked	1.73	-4.4%	0.077	1.3%
	Living Donor spiked	1.81			1.1%
Medium / high Positive	Post-Mortem spiked	2.94	-4.2%	0.121	n.a.
	Living Donor spiked	3.07			

* Paul Ehrlich Institute - Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, Anti-HCV-Assays, HBsAg and Anti-HBc Assays for Use with Cadaveric Samples - 08/05/2014

16. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

Diagnostic specificity was assessed by testing 1999 specimens from an anti-HDV negative population (blood donors, HBsAg positive carriers, pregnant women, patients affected by non-HDV infectious diseases, subjects with autoimmune diseases or other diseases

related to disorders of the immune system, subjects from a HDV diagnostic routine). 14 positive results were observed in the population studied: diagnostic specificity 99.30% (95% Confidence Interval: 98.83 – 99.62%).

Populations	Number of cases	LIAISON® XL MUREX Anti-HDV Reactive samples	Diagnostic Specificity %	Diagnostic Specificity 95% CI
Blood Donors	1009	7	99.31 (1002/1009)	98.58 – 99.72%
HBsAg positive	427	2	99.53 (425/427)	98.32 – 99.94%
HDV diagnostic routine	190	1	99.47 (189/190)	97.10 – 99.98%
Potential cross-reactive	252	1	99.60 (251/252)	97.81 – 99.99%
Pregnant women	121	3	97.52 (118/121)	92.93 – 99.49%
Overall	1999	14	99.30 (1985/1999)	98.83 – 99.62%

Diagnostic sensitivity was assessed by testing 174 specimens from an expected anti-HDV positive population collected in different laboratories in a European country. No negative results were observed in the population studied: diagnostic sensitivity 100% (95% Confidence Interval: 97.90 – 100%).

Populations	Number of cases	LIAISON® XL MUREX Anti-HDV Reactive samples	Diagnostic Sensitivity %	Diagnostic Sensitivity 95% CI
HBsAg positive	7	7	100 (7/7)	59.04 – 100%
Selected Anti-HDV pos	100	100	100 (100/100)	96.38 – 100%
HDV diagnostic routine	67	67	100 (67/67)	94.64 – 100%
Overall	174	174	100 (174/174)	97.90 – 100%

The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.
EN - 200/008-824, 08 - 2026-03-23

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.

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