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LIAISON® XL MUREX HBsAg (REF 317250)

1. INTENDED PURPOSE

The LIAISON® XL MUREX HBsAg assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of Hepatitis B surface Antigen (HBsAg) in human serum and plasma samples including specimens collected post-mortem (non-heart beating).

The assay is intended as an aid in the diagnosis of HBV infection in individuals with or without symptoms of hepatitis. It is also intended as a screening test for blood and hemocomponents donors as well as for organ, tissue and cells post-mortem donors.

The test has to be performed on the LIAISON® XL Analyzer only.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis is an inflammatory disease of the liver that can severely damage the organ. The disease can result from non-infectious causes or from infectious viral and bacterial agents, with viral hepatitis B with an endemic worldwide distribution. More than 2 billion people have serologic evidence of a past or present hepatitis B virus (HBV) infection and around 250 million people are chronically infected and at risk of developing HBV-related liver disorders.

HBV is transmitted through contact with blood or other body fluids from an infected person ^(1, 6) or by perinatal, percutaneous, parenteral and sexual exposure.

HBV infection can lead to a self-limiting acute hepatitis B disease, with acute inflammation and hepatocellular necrosis ⁽¹⁾. Spontaneous recovery occurs in more than 95% of cases ^(1, 12), however, a small subset of patients may develop acute liver failure due to fulminant hepatitis ⁽⁸⁾. After 6 months of persistent infection, patients develop chronic hepatitis, which progresses through five main clinical phases: the immune-tolerant, immune-active, immune-control, immune-escape and HBsAg-clearance phase. ⁽⁶⁾

HBV is a small, enveloped and primarily hepatotropic DNA virus, member of the Hepadnaviridae family. Its small genome (3.2 kb) contains four open reading frames encoding 7 proteins: the HBV e antigen (HBeAg), the HBV core antigen (HBcAg), the reverse transcriptase, the transcriptional regulator HBV x antigen (HBx) and three surface envelope glycoproteins (PreS1, PreS2 and hepatitis B surface antigen) ⁽¹⁾.

The covalently closed circular DNA (cccDNA) is used as a template for viral transcription, including the production of HBs proteins, which greatly exceeds the demand for virion formation and are released as non-infectious subviral particles. The HBs protein has the determinant antigenic component for the activation of the host immunity and its serum concentration depends on the amount of hepatitis B cccDNA genomes in hepatocytes, their transcriptional activity and the host immune response against the virus ^(2, 3).

HBsAg is the first serological marker to appear, within 10 weeks after the exposure to the virus ⁽³⁾. During acute hepatitis, HBeAg and HBV DNA levels are initially high and recovery is indicated by the disappearance of these two markers, the seroconversion to anti-HBe and anti-HBs and HBsAg clearance ^(6, 9). The determination of serum HBsAg is recommended six months after diagnosis, even if liver function tests are normal ^(10, 14), to distinguish an acute from a chronic infection.

HBsAg detection is recommended by many organizations, such as WHO, EASL and CDC ^{1, 4, 7)} and it is used for the initial assessment of hepatitis B infection, for either screening asymptomatic high-risk patients or diagnosing patients with symptoms. A positive HBsAg result is usually followed by additional tests which include HBV markers (HBeAg, anti-HBe, HBV DNA) and liver function tests (ALT, fibrosis markers, liver ultrasound, liver biopsy) ^(1, 2, 6, 11, 13).

Screening of blood and blood products obtained for transfusion is an important preventive measure against chronic hepatitis B and HBsAg assays are the first-line methods ^(5, 8, 9, 16). Sensitive and specific immunoassays are recommended, and all HBsAg-positive blood products should be considered to be at high risk of HBV transmission and should not be used for transfusion ⁽¹⁵⁾.

Similar to blood donations, donated organs also need to be tested for agents that may cause organ transplant-mediated transmission of infections. HBsAg testing is recommended by the European Directorate for the Quality of Medicines and Health Care (EDQM, Europe). A positive HBsAg result indicates that HBV transmission will occur with organ transplantation and HBsAg-positive donors may only be considered for HBV-positive recipients or some other exceptional scenarios ⁽¹⁷⁾.

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of HBsAg is a direct sandwich chemiluminescence immunoassay (CLIA). Adequate detection of mutants and genotypes is assured by mouse monoclonal antibodies directed to highly conserved epitopes of HBsAg inner region that can detect HBsAg when used in combination with a complex detergent mixture.

A mixture of mouse monoclonal antibodies is used for coating magnetic particles (solid phase) and a different mixture of mouse monoclonal antibodies directed to different epitopes is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, HBsAg present in calibrators, samples or controls binds to the solid phase. During the second incubation, the antibody conjugate reacts with HBsAg 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 directly proportional to HBsAg concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	[SORB]	Magnetic particles ($\geq 0.25\%$ solid) coated with mouse monoclonal antibodies to HBsAg (approx. 40 $\mu\text{g/mL}$), biotinylated BSA, streptavidin, BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (3.0 mL)	[CAL1]	Low levels of recombinant HBsAg (obtained in mammalian cells) (approx. 1 S/CO), BSA, phosphate buffer, EDTA, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentration is referenced to an in house antigen preparation.
Buffer L (28 mL)	[BUF L]	Non-specific IgG (mouse polyclonal), casein, TRIS buffer, EDTA, detergents, 0.1% ProClin™ 300.
Conjugate (2 x 23 mL)	[CONJ]	Mouse monoclonal antibodies to HBsAg conjugated to an isoluminol derivative (approx. 0.95 $\mu\text{g/mL}$), human and animal sera, non-specific IgG (mouse polyclonal), BSA, phosphate buffer, detergents, 0.2% ProClin™ 300, preservatives.
Number of tests		200

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® XL Cuvettes ([REF] X0016).
LIAISON® XL Disposable Tips ([REF] X0015) or
LIAISON® Disposable Tips ([REF] X0055).
LIAISON® XL Starter Kit ([REF] 319200) or
LIAISON® EASY Starter Kit ([REF] 319300).
LIAISON® Wash/System Liquid ([REF] 319100).
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LIAISON® XL Waste Bags ([REF] X0025).
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Additional required materials

LIAISON® XL MUREX Control HBsAg (negative and positive) (**[REF]** 317251).
LIAISON® XL MUREX HBsAg Confirmatory (**[REF]** 317252).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

For Laboratory Professional Use Only.

All materials used to produce the components 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, except for the positive control, which is reactive for HBsAg. The hepatitis B surface antigen has been heat treated (60°C for 10 hours) during the manufacturing process. Nevertheless, complete inactivation should not be assumed.

However, as no test method can offer absolute assurance of the absence of pathogens, all specimens of human origin should be considered potentially infectious and handled with care.

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.

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. Waste must be handled with care and disposed of in compliance with 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® 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 after the expiration date given on the label.

Strict adherence to the instructions is necessary to obtain reliable results.

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

REAGENTS:	CAL1	CONJ	BUFL
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 Aquatic chronic 3 H412	Acute Tox. 3 H331 Skin Irrit. 2 H315 Eye Dam. 1 H318 Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning	Warning	Danger
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	 GHS07 Exclamation mark	  GHS05 Corrosion GHS06 Skull and cross bones
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.	H331 Toxic if inhaled. H315 Causes skin irritation. H318 Causes serious eye damage. 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. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P362+P364 Take off all contaminated clothing and wash it before reuse. P273 Avoid release to the environment.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P362+P364 Take off all contaminated clothing and wash it before reuse. P273 Avoid release to the environment.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 IF IN EYES rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P310 Immediately call a POISON CENTER or doctor/physician. P403+P233 Store in a well-ventilated place. Keep container tightly closed
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	Mixture 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). Octylphenol polyethoxyethanol, Triton™ X-705	Mixture 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).	N-Lauroylsarcosine sodium salt Mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7]; 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300); Poly(oxy-1,2-ethanediyl),.alpha.-[4-(1,1,3,3-tetramethylbutyl)phenyl]-.omega.- hydroxy (Triton™ X-100). N,N-Dimethylformamide N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (SB-16)

Pursuant to EC Regulation 1272/2008 (CLP), **SORB** is labeled as EUH210 safety data sheets available on request.

For additional information see 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 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 particles 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 calibrator in particular (position two following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foaming 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® XL Analyzer is equipped with a built-in solid-state magnetic device that 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 using it. 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 Control HBsAg instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles. Refer to Reagent Integral Preparation for resuspension instructions.

- **Sealed:** Stable at 2-8°C until the expiry date.
- **Opened on board or at 2-8°C:** up to twelve (12) weeks.
- Use storage rack provided with the LIAISON® XL Analyzer for upright storage of reagent integral.
- Do not freeze.
- Keep upright for storage to facilitate later 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 (without and with gel- SST);
- sodium and lithium heparin plasma;
- K₂-EDTA plasma;
- sodium citrate plasma;
- potassium oxalate plasma;
- ACD plasma;
- CPDAplasma.

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 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:

- 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 7 freeze-thaw cycles, however multiple freeze-thaw cycles should be avoided;
- up to 4 months 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.

Cadaveric specimens should be stored following same indications than for living donors.

Additional volume is required for samples that are repeatedly reactive and require confirmatory testing. Refer to the LIAISON® XL MUREX HBsAg Confirmatory assay (REF 317252) Instruction for use-section 8.

The minimum volume required for a single determination using one single tube is 300 µL specimen (150 µL specimen + 150 µL dead volume).

10. CALIBRATION

Assay of calibrator contained in the reagent integral allows the analyzer to set the assay cut-off.

Each calibration 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 eight (8) weeks before.
- Each time a new lot of integral is used.
- Control values lie outside the expected ranges.
- The analyzer has been serviced.

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.

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 instruction.

The analyzer operations are as follows:

1. Dispense calibrators, controls or specimens, coated magnetic particles and buffer L into the reaction cuvettes
2. Incubate.
3. Dispense conjugate into the reaction cuvettes.
4. Incubate.
5. Wash with Wash/System liquid.
6. Add the Starter Reagents and measure the light emitted.

Due to the presence of detergents in the LIAISON® XL MUREX HBsAg reagents, foam may be generated in the Liquid Waste container. If this happens, in order to avoid overflow of the foam from the container it is advisable to empty the waste container when the level of the liquid is approximately half of the capability of the container or alternatively to employ a silicone based antifoam, to be added into the Liquid Waste container when it is empty and hypochlorite is added.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® XL MUREX Control HBsAg ([REF] 317251):

- (a) at least once per day of use,
- (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 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 being used. Appropriate value ranges should then be established for the quality control materials used.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates HBsAg level expressed as signal to cut-off (S/CO) and grades the results. For details, refer to the analyzer operator's manual.

The interpretation of results for the LIAISON® XL MUREX HBsAg is as follows:

- Cut-off value of a 1.00 S/CO determines whether a sample has detectable levels of HBsAg.
- **Non-Reactive:** Samples with HBsAg levels below the S/CO value of 1.00 are considered Non-reactive and presumed negative for HBsAg.
- **Reactive:** Samples with HBsAg levels at initial testing equal to or above the S/CO value of 50.0 are considered Reactive and presumed positive for HBsAg, and no further testing is required. Samples with HBsAg levels equal to or above a S/CO value of 1.00 but less than 50 are considered initially Reactive and presumed positive for HBsAg.

Initially Reactive samples with an S/CO value lower than 50 must be retested in duplicate. Samples with HBsAg levels below the S/CO value of 1.00 in both replicates at the retest are considered Non-Reactive and presumed negative for HBsAg. Samples that are repeatedly equal to or above a S/CO of 1.00 (i.e. at least 2 out of 3 results) are considered repeatedly Reactive and presumed positive for HBsAg. Samples that are repeatedly Reactive for HBsAg should be evaluated with supplementary investigation, such as other HBV markers or HBV DNA or LIAISON® XL MUREX HBsAg Confirmatory ([REF] 317252).

14. LIMITATIONS OF THE PROCEDURE

- 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.
- Specimens should be kept at room temperature only for the amount of time required for handling and preparation.
- This test is suitable only for investigating single samples, not for diluted specimens, sample pools or heat-inactivated specimens
- Falsely reactive results may be obtained with any diagnostic test. Two kinds of falsely reactive result may be observed with LIAISON® XL MUREX HBsAg test: non-reproducibly reactive results and non-specifically reactive results.
- Specimens from individuals recently vaccinated against HBV may score transiently positive for HBsAg because it is present in the vaccine. Reactivity to vaccine may vary with different manufacturers' tests.
- Non-specifically reactive results may be observed in most highly sensitive immunoassays. For instance, 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. Non-specifically reactive results, however, are eventually correctly classified by LIAISON® XL MUREX HBsAg Confirmatory ([REF] 317252).
- 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 judgement. A full differential diagnostic work-up for the diagnosis of hepatitis B and related clinical conditions includes examination of the patient's immune status and clinical history.
- Specimens from patients receiving therapeutic doses of Biotin (Vitamin H, B7 or B8) may interfere in immunoassays based on biotinylated reagents. Interference was not observed testing Biotin serum concentration up to 3500 ng/mL with LIAISON® XL MUREX HBsAg assay (for details, refer to §15.1).
- 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.
- Do not heat-inactivate sera.

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 that the assay performance was not affected by anticoagulants (lithium and sodium heparin, sodium citrate, potassium EDTA, potassium oxalate, ACD and CPDA) and by each substance listed below, at the indicated concentration.

Substances	Tested concentrations	Substances	Tested concentrations
Triglycerides	3000 mg/dL	Vitamin C	300 mg/L
Hemoglobin	1000 mg/dL	Vitamin D	100 ng/mL
Unconjugated bilirubin	40 mg/dL	Interferon alpha 2a	6000 IE/mL
Conjugated bilirubin	40 mg/dL	Interferon alpha 2b	6000 IE/mL
Albumin	6000 mg/dL	Interferon alpha 1b	6000 IE/mL
Cholesterol	400 mg/dL	Ibuprofen	21.9 mg/dL
Vitamin H (Biotin)	3500 ng/mL	Entecavir	0.5 mg/L
Total Protein (high)	150 g/L	Tenofovir	0.0978 mg/dL
Total Protein (low)	50 g/L	Lamivudine	300 mg/L
Telbivudine	600 mg/L	Adefovir dipivoxil	10 mg/L
Acetaminophen	15.6 mg/dL		

Cross-reactions.

The LIAISON® XL MUREX HBsAg assay was evaluated for potential cross-reactivity with other viruses that may cause symptoms similar to HBV infection (HAV, HCV), other organisms that may cause infectious disease (CMV, HSV, EBV, *T. cruzi*, *T. pallidum*, HIV, HTLV) and from other conditions that may result from atypical immune system activity (i.e. rheumatoid factor, anti-nuclear antibodies, HAMA). In the study, 384 expected negative samples were found to be non-reactive with the LIAISON® XL MUREX HBsAg assay while one (1) sample was found to be non-reactive with the reference assay and reactive with the LIAISON® XL MUREX HBsAg assay. Conclusion: No significant interference is demonstrated by the medical conditions presented in this comparison study.

Organism / Condition	Expected negative (N)	LIAISON® XL MUREX HBsAg	
		Non-reactive	Reactive
Anti-nuclear antibodies (ANA)	14	14	0
Non-viral liver diseases (i.e. Auto-immune hepatitis)	15	15	0
<i>C. trachomatis</i> (anti-chlamydia positive)	15	14	1
CMV (anti-CMV positive)	13	13	0
EBV (anti-EBV positive)	15	15	0
HSV (anti-HSV positive)	15	15	0
Fatty liver disease	14	14	0
HAMA	10	10	0
Hemodialysis patient	15	15	0
Hepatitis A Virus (anti-HAV positive)	11	11	0
Hepatitis C Virus (anti-HCV positive)	15	15	0
Hepatocellular carcinoma	12	12	0
HIV-1 (anti-HIV-1 positive)	2	2	0
HIV-2 (anti-HIV-2 positive)	12	12	0
HIV (anti-HIV positive)	15	15	0
HTLV-1/2 (anti-HTLV positive)	15	15	0
IgG monoclonal gammopathy	16	16	0
IgM monoclonal gammopathy	4	4	0
Influenza vaccine recipients	15	15	0
Multiparous pregnancies	15	15	0
Multiple myeloma	14	14	0
Multiple transfusion recipients	15	15	0
<i>N. gonorrhoeae</i> (anti-Neisseria positive)	12	12	0
Pregnancy 1 st trimester	15	15	0
Pregnancy 2 nd trimester	15	15	0
Pregnancy 3 rd trimester	15	15	0
Rheumatoid Factor	15	15	0
<i>T. pallidum</i> (anti-treponema positive)	12	12	0
<i>T. cruzi</i> (anti-T. cruzi positive)	14	14	0

15.2. Analytical sensitivity

In order to determine sensitivity of LIAISON® XL MUREX HBsAg assay, the HBsAg concentration which corresponds to the measured signal of the cutoff value was read off the curves of serial dilutions of WHO 3rd International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2) in human negative serum and plasma matrices.

The analytical sensitivity of LIAISON® XL MUREX HBsAg assay at cutoff level is approximately 0.05 IU/mL.

15.3. Precision with LIAISON® XL

A twenty (20) day reproducibility/precision study was performed by using a coded panel that was prepared by either spiking or diluting samples as necessary to obtain negative, low positive and mid positive samples. Kit Control sets were also included in the 20-day study. The panel samples and kit controls were tested on three (3) LIAISON® XL MUREX HBsAg kit lots in two (2) replicates per run, two (2) runs per day for twenty (20) operating days on one (1) LIAISON® XL Analyzer. The CLSI document EP5-A3 was consulted in the preparation of the testing protocol.

LIAISON® XL MUREX HBsAg Assay All 3 Lots Combined												
Sample ID	N	Mean (S/CO)	Repeatability		Between-Run		Between-Day		Between-Lots		Within Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl lot 1	240	0.45	0.026	5.8%	0.025	5.6%	0.029	6.5%	0.039	8.6%	0.061	13.5%
Neg Ctrl lot 2	240	0.46	0.029	6.4%	0.018	4.0%	0.033	7.2%	0.034	7.5%	0.058	12.8%
Neg Ctrl lot 3	240	0.46	0.028	6.2%	0.016	3.4%	0.034	7.5%	0.039	8.4%	0.061	13.3%
Pos Ctrl lot 1	240	2.18	0.078	3.6%	0.046	2.1%	0.095	4.4%	0.107	4.9%	0.169	7.8%
Pos Ctrl lot 2	240	2.03	0.069	3.4%	0.040	2.0%	0.092	4.5%	0.072	3.5%	0.141	7.0%
Pos Ctrl lot 3	240	1.99	0.066	3.3%	0.038	1.9%	0.090	4.5%	0.067	3.4%	0.136	6.8%
HBS1U10	240	0.43	0.034	7.9%	0.038	8.9%	0.025	5.8%	0.019	4.3%	0.060	13.9%
HBS1U11	240	0.81	0.039	4.8%	0.023	2.9%	0.043	5.3%	0.028	3.4%	0.069	8.4%
HBS1U12	240	0.80	0.033	4.1%	0.021	2.6%	0.051	6.3%	0.030	3.7%	0.071	8.8%
HBS1U13	240	0.80	0.031	3.9%	0.023	2.9%	0.044	5.5%	0.031	3.9%	0.066	8.3%
HBS1U14	240	1.25	0.041	3.3%	0.027	2.1%	0.091	7.3%	0.050	4.0%	0.114	9.2%
HBS1U15	240	1.24	0.043	3.5%	0.038	3.1%	0.091	7.3%	0.049	3.9%	0.118	9.5%
HBS1U16	240	1.25	0.043	3.4%	0.038	3.0%	0.082	6.5%	0.046	3.7%	0.110	8.8%
HBS1U17	240	3.36	0.088	2.6%	0.086	2.6%	0.127	3.8%	0.144	4.3%	0.228	6.8%
HBS1U18	240	3.40	0.063	1.8%	0.064	1.9%	0.150	4.4%	0.142	4.2%	0.225	6.6%
HBS1U19	240	3.38	0.062	1.8%	0.070	2.1%	0.136	4.0%	0.120	3.5%	0.204	6.0%
HBS1U20	240	1.18	0.039	3.3%	0.022	1.8%	0.046	4.0%	0.041	3.5%	0.077	6.5%

A 5-day reproducibility/precision study was conducted at three (3) external laboratories. Each site used a different lot of LIAISON® XL MUREX HBsAg assay. The coded panel used in the 5-day study was the same panel used in the 20-day study. The coded panel was tested at all three (3) sites, using six (6) replicates per run in one (1) run per day for five (5) operating days. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol. The mean, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens across sites.

LIAISON® XL MUREX HBsAg Assay 5 Day Multi-Site / Multi-Lot												
Sample ID	N	Mean (S/CO)	Repeatability		Between-Day/Runs		Within Laboratory		Between Sites/Lots		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl (all 3 lots)	90	0.422	0.036	8.6%	0.032	7.7%	0.049	11.5%	0.049	11.6%	0.069	16.3%
Pos Ctrl (all 3 lots)	90	2.066	0.046	2.2%	0.045	2.2%	0.064	3.1%	0.058	2.8%	0.086	4.2%
HBS1U10	90	0.380	0.025	6.5%	0.026	6.8%	0.036	9.4%	0.026	6.8%	0.044	11.6%
HBS1U11	90	0.815	0.028	3.5%	0.034	4.2%	0.044	5.4%	0.047	5.8%	0.065	7.9%
HBS1U12	90	0.807	0.032	4.0%	0.033	4.1%	0.046	5.8%	0.048	5.9%	0.067	8.2%
HBS1U13	90	0.801	0.035	4.3%	0.037	4.6%	0.051	6.3%	0.033	4.1%	0.060	7.5%
HBS1U14	90	1.264	0.036	2.9%	0.034	2.7%	0.050	3.9%	0.042	3.4%	0.065	5.2%
HBS1U15	90	1.261	0.034	2.7%	0.045	3.6%	0.056	4.5%	0.040	3.2%	0.069	5.5%
HBS1U16	90	1.273	0.039	3.0%	0.041	3.2%	0.056	4.4%	0.029	2.3%	0.063	5.0%
HBS1U17	90	3.570	0.072	2.0%	0.057	1.6%	0.092	2.6%	0.129	3.6%	0.158	4.4%
HBS1U18	90	3.552	0.063	1.8%	0.044	1.2%	0.077	2.2%	0.115	3.2%	0.138	3.9%
HBS1U19	90	3.568	0.074	2.1%	0.058	1.6%	0.094	2.6%	0.119	3.3%	0.152	4.2%
HBS1U20	90	1.221	0.037	3.1%	0.038	3.1%	0.053	4.3%	0.055	4.5%	0.076	6.3%

15.4. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than actually present.

Analysis of saturation effect was evaluated by testing four (4) high-titred serum samples positive for HBsAg All samples resulted in estimated concentration values above the assay range that would be expected with high-titred samples, indicating no sample misclassification and with no high-dose saturation effect observed.

No high-dose hook effect was observed up to a S/CO value of 3322.

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. Twenty (20) post-mortem samples were tested as unspiked and spiked at two levels: level 1 (low positive) and level 2 (medium/high positive). The same procedure was performed with the same number of normal human samples 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. The results refer to the group of investigated samples and are not guaranteed specifications, as differences may exist between laboratories and locations.

Repeatability was assessed using one post-mortem and one living donor specimens, spiked up to a low-level of reactivity with a human serum reactive for Hepatitis B surface Antigen (HBsAg positive). 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 2.4% for the cadaveric specimen and 2.3% 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 (S/CO)	Recovery (%) post-mortem / living donors	CV% 6 replicates
Level 1	Post-Mortem spiked	1.52	-5.4%	2.4%
	Living Donor spiked	1.44		2.3%
Level 2	Post-Mortem spiked	2.35	6.7%	n.a.
	Living Donor spiked	2.51		

* 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. EXPECTED VALUES

Diagnostic specificity and sensitivity were estimated in accordance with Common Specification (CS) published on July 5, 2022 (COMMISSION IMPLEMENTING REGULATION (EU) 2022/1107 of 4 July 2022). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

16.1. Diagnostic specificity

A study was performed on a total of 5400 serum and plasma specimens collected in three blood donation centers (including 100 specimens from first-time donors). Specimens tested were expected negative samples from an unselected blood donor population with zero prevalence of HBV infection. The assay shows diagnostic specificity above 99.5% (95% confidence interval: 99.8 -100%). Additional specimens were also tested, randomly selected from hospitalized patients. Data of these studies are summarized in Table I (95% CI = 95% confidence interval). Positive specimens were confirmed by a reference CE-marked kit.

Table I - Diagnostic specificity.

Population	Number of cases	Initially reactive samples, No.	Repeat reactive samples, No.	Confirmed positive samples, No.	Diagnostic specificity, %	Diagnostic specificity, 95% CI
Blood donors	5400	10	4	0	99.93 (5396/5400)	99.8-100.0
Hospitalized patients	400	5	5	4	99.75 (395/396)	98.6-100.0

The assay performance in terms of specificity is comparable with tests currently available on the CE market, met CS requirements, and represents the state of the art for HBsAg detection.

An additional study performed versus a comparator method on following populations showed performance summarized in Table II (95% CI = 95% confidence interval).

Table II - Diagnostic specificity on additional populations.

Population	Number of cases	Initially reactive samples, No.	Repeat reactive samples, No.	Confirmed positive samples, No.	Diagnostic specificity, %	Diagnostic specificity, 95% CI
Dialysis patients	100	0	0	0	100 (100/100)	96.3-100.0
Pregnant women	800	5	5	4	99.87 (795/796)	99.3-100.0
High risk	1147	8	7	4	99.91 (1138/1139)	99.5-100.0

16.2. Diagnostic sensitivity

Diagnostic sensitivity was assessed by testing 452 specimens from preselected HBsAg-positive patients. Diagnostic sensitivity of this study is 100% (95% confidence interval: 99.2-100.0%). Besides, results obtained are substantially comparable with those expected on four commercially available panels encompassing different HBsAg subtypes (ad and ay) and genotypes.

Seroconversion panel Performance

In an additional study the ability of the LIAISON® XL MUREX HBsAg assay to detect HBsAg was evaluated by testing sequentially-collected specimens belonging to 30 seroconversion panels from donors who seroconverted over the course of their donation history. Commercially available, precharacterized panels for HBV antigens were used, each starting with a negative bleed and exhibiting narrow bleeding intervals. The panels were also tested by commercially available HBsAg comparator assays. The results show that the LIAISON® XL MUREX HBsAg assay detected HBsAg one bleed earlier in three (3) out of 30 panels. The comparator assay detected HBsAg one bleed earlier in three (3) out of 30 panels. Both assays exhibited equivalent HBsAg detection in 24 out of 30 panels. The test diagnostic sensitivity in the detection of HBV early infection is therefore substantially equivalent to the reference assay.

16.3. HBsAg mutant detection

A panel of ten (10) recombinant mutants were tested with the LIAISON® XL MUREX HBsAg and a comparator CE-marked assay to determine correct antigenic detection of the HBsAg structure. The mutants contained important epitope clusters within amino acids 100-160, that includes the "a determinant region" (amino acids 124-147), the most important target for serological diagnosis. The recombinant mutants were diluted in HBsAg negative human serum to yield a low positive sample. All 10 recombinant mutants were recognized with LIAISON® XL MUREX HBsAg.

Sample ID	Mutation	Comparator HBsAg assay	LIAISON® XL MUREX HBsAg assay
Mutant-01	T123N	Reactive	Reactive
Mutant-02	T123N-T124S	Reactive	Reactive
Mutant-03	P142L-F/Y143H-D144E-G145-R	Reactive	Reactive
Mutant-04	I110R-SS117I-G119R-T123N	Reactive	Reactive
Mutant-05	122+DT	Reactive	Reactive
Mutant-06	122+DT-G145R	Reactive	Reactive
Mutant-07	G145R	Reactive	Reactive
Mutant-08	D114A	Reactive	Reactive
Mutant-09	P142L-G145R	Reactive	Reactive
Mutant-10	P142S-G145R	Reactive	Reactive

16.4. HBsAg genotypes detection

Thirty (30) specimens from commercially available HBsAg performance panels containing the most common hepatitis B surface antigen genotypes (A through H) were tested to assess the performance of the assay. All of the thirty specimens resulted HBsAg reactive with LIAISON® XL MUREX HBsAg.

16.5. Pediatric samples

Pediatric samples were tested to determine if these types of samples provide equivalent results to adult human serum.

A total of thirty (30) negative pediatric patient samples were used for this study. The pediatric samples encompassed the age range of two (2) years to twenty-one (21) years. Ten (10) negative pediatric samples were spiked with HBsAg high positive sample to obtain high negative samples. Ten (10) pediatric samples were spiked with HBsAg high positive sample to obtain low positive samples. Ten (10) pediatric samples were spiked with HBsAg high positive sample to obtain moderate positive samples. Adult negative pool samples were used as controls, by spiking with HBsAg high positive sample to achieve the same three (3) levels of samples: high negative, low positive and moderate positive samples. Averaged results for each pediatric sample were compared to results obtained on adult samples. The results of the study show that pediatric samples react in the same manner as adult samples.

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 shall be reported to DiaSorin Italia S.p.A. UK Branch and the Competent Authority of the EU Member State in which the user and/or the patient is established.

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