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LIAISON® XL MUREX HBsAg Confirmatory (REF 317252)

1. INTENDED PURPOSE

The LIAISON® XL MUREX HBsAg Confirmatory is an *in vitro* neutralization assay for confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma samples found repeatedly reactive for HBsAg by the LIAISON® XL MUREX HBsAg (REF 317250).

2. SUMMARY AND EXPLANATION OF THE TEST

With the discovery and correlation of the hepatitis B surface antigen (HBsAg) to type B viral hepatitis, the problem of detecting potentially infectious blood units became critically important. Since then, test methods with increasing sensitivity and specificity have been developed to allow for HBsAg detection in donor and patient populations. In spite of the high specificity achieved with these test methods, the possibility exists that falsely reactive results may be encountered due to the presence of non-specific interfering substances, artifacts in the reagents, or the type of methodology used. To reduce this possibility and avoid falsely reactive results in diagnosing HBV infection as well as the discarding of useful blood units, neutralization tests were developed to ensure that HBsAg-reactive results are caused by the presence of the surface antigen and not by non-specific interference.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® XL MUREX HBsAg Confirmatory is based on the principle of binding inhibition or neutralization of binding activity. A neutralizing reagent, containing goat antibodies to HBsAg is added to one aliquot of each specimen found repeatedly reactive (neutralized aliquot). As a control procedure, anti-HBs negative human serum is added to the other aliquot (non-neutralized aliquot). If the neutralizing reagent has been added to a sample containing HBsAg, the antibodies in the neutralizing reagent will bind to the HBsAg, forming an antigen-antibody complex. If the neutralizing reagent has been added to a sample containing an interfering substance, the antibodies in the neutralizing reagent will not bind to the interfering substance. Dilution may be used to ensure neutralization of samples with elevated concentrations of HBsAg that may exceed the potency of the LIAISON® XL MUREX HBsAg Confirmatory neutralizing reagent. In the confirmation procedure, each reactive sample is incubated in the presence of a solid matrix coated with mouse monoclonal antibodies to HBsAg, whereupon either a solid-matrix antibody-antigen-antibody complex or a solid-matrix antibody interfering substance complex is formed.

Next, the antibody conjugate from the screening kit, which contains antibodies to HBsAg, is added. If an antibody-antigen-antibody complex is present, the antibody conjugate will bind to the complex only partially. If an antibody-interfering substance complex is present, the antibody conjugate will bind non-specifically to the interfering substance.

Therefore, if the repeatedly HBsAg-reactive result was caused by the presence of HBsAg in the sample, the antibodies in the neutralizing reagent will neutralize the reactivity of HBsAg by inhibiting the binding of the antibody conjugate. The resulting signal will be lower than the signal obtained in the aliquot to which anti-HBs negative human serum was added.

Conversely, if the repeatedly HBsAg-reactive result was caused by the presence of an interfering substance, the antibodies in the neutralizing reagent will not neutralize the interfering substance, and the antibody conjugate will bind to it non-specifically. The resulting signal will be similar to that of the aliquot to which anti-HBs negative human serum was added.

If the signal of the neutralized aliquot is significantly lower than the signal of the non-neutralized aliquot, the presence of HBsAg in the sample is confirmed.

4. MATERIALS PROVIDED

Reagent integral

Neutralizing solution (Anti-HBs) (1 vial, 0.7 mL)	Anti-HBs	Goat plasma containing at least 20,000 mIU/mL anti-HBs antibodies, human defibrinated plasma negative for HBsAg, 0.2% ProClin™ 300, preservatives, an inert red dye (ready to use).
Specimen Diluent (1 vial, 16 mL)	DIL SPE	Human serum negative for HBsAg, anti HIV 1/2, anti-HCV, anti-HBs, 0.2% ProClin™ 300, preservatives (ready to use).
Number of tests		20 specimens including controls.

ProClin™ is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow. All reagents are supplied ready to use.

5. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

LIAISON® XL MUREX HBsAg (REF 317250).

LIAISON® XL MUREX Control HBsAg (REF 317251).

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).

LIAISON® XL Waste Bags (REF X0025).

6. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

For Laboratory Professional Use Only.

Visually inspect the 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.

Do not mix reagents or exchange components from kits with different lot numbers.

All serum and plasma units 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. As, however, 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.

LIAISON® XL MUREX HBsAg and LIAISON® XL MUREX Control HBsAg are not kit lot specific and may be safely interchanged even from different lots. The performance characteristics of LIAISON® controls have not been established for any other assays or instrument platforms different from LIAISON® XL

When the presence of HBsAg cannot be confirmed by neutralization assay or the infection is however suspected it is suggested to evaluate individual's HBV infection with supplementary investigation, such as other HBV serological markers or HBV DNA. For instance, when HBsAg is present at low levels, in conjunction with a non-specific reactivity, the neutralization may be incomplete, and it is possible (as with any neutralisation assay) that some mutated forms of HBsAg may fail to be inhibited.

Diagnostic performance of LIAISON® XL MUREX HBsAg Confirmatory (REF 317252) have been established only when used in combination with LIAISON® XL MUREX HBsAg (REF 317250) and LIAISON® XL MUREX Control HBsAg (REF 317251).

7. 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.

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 labelled as follows:

REAGENTS:	Anti-HBs, DIL SPE
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).

For additional information see Safety Data Sheets available on www.diasorin.com.

8. STORAGE AND STABILITY OF REAGENTS

Upon receipt, the reagents must be stored at 2-8°C. Do not freeze. When the reagents are stored sealed, they are stable at 2-8°C up to the expiry date. Once opened the reagents are stable for eight (8) weeks when properly stored at 2-8°C between two successive uses. Avoid bacterial contamination. The reagents should not be used past the expiry date indicated on the vial labels.

9. SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma may be used. Several anticoagulants have been tested and may be used with this assay.
For specimen collection and preparation refer to Instruction for Use of LIAISON® XL MUREX HBsAg (REF 317250).
The minimum volume of specimen required for this confirmatory assay is 700 µL for reactive samples (i.e. S/CO value < 50).

10. ASSAY PROCEDURE

Test the LIAISON® XL Murex HBsAg negative and positive controls (non-treated) with each specimen run. The Positive control must also be tested following the same Specimen treatment procedure used for repeatedly reactive patient samples with S/CO lower than 50.

Specimen treatment

Mix 330 µL specimen and 33 µL anti-HBs antibodies in one tube (neutralized aliquot) as well as 330 µL specimen and 33 µL LIAISON® XL MUREX HBsAg Confirmatory specimen diluent in another tube (non-neutralized aliquot).
Incubate the specimens for minimum one hour at room temperature (20-25°C). Treat controls and specimens in parallel.

Assay procedure For Use with the LIAISON® XL MUREX HBsAg (REF 317250) on the LIAISON® XL Analyzer

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 of LIAISON® XL MUREX HBsAg test into reaction cuvettes (as required).
2. Dispense one non-treated negative control, one non-treated positive control, and two positive controls (neutralized and non-neutralized aliquots) of LIAISON® XL MUREX HBsAg control set into the reaction cuvettes.
3. Dispense neutralized and non-neutralized aliquots of each specimen into the reaction cuvettes.
4. Dispense coated magnetic particles and buffer L into the reaction cuvettes.
5. Incubate.
6. Dispense conjugate into the reaction cuvettes.
7. Incubate
8. Wash with Wash/System liquid.
9. Add the Starter Reagents and measure the light emitted.

11. QUALITY CONTROL

Quality control is suggested once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. Non-treated LIAISON® XL MUREX Control HBsAg (REF 317251) (Negative and Positive) must be tested in every LIAISON® XL MUREX HBsAg Confirmatory run.
Non-treated negative and positive controls may not be run if a LIAISON® XL MUREX HBsAg test has been already performed on that day.
LIAISON® controls are available for internal quality control. Whenever controls lie outside the expected ranges, calibration should be repeated, and controls retested.
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 of the materials used.

12. LIMITATIONS OF THE PROCEDURE

When the presence of HBsAg cannot be confirmed by neutralization assay or the infection is however suspected it is suggested to evaluate individual's HBV infection with supplementary investigation, such as other HBV serological markers or HBV DNA. For instance, when HBsAg is present at low levels, in conjunction with a non-specific reactivity, the neutralization may be incomplete and it is possible (as with any neutralization assay) that some mutated forms of HBsAg may fail to be inhibited.

13. CALCULATION OF RESULTS

The percentage of neutralization is given by the following formula:

$$\frac{\text{(RLU for non-neutralized aliquot - RLU for neutralized aliquot)}}{\text{(RLU for non-neutralized aliquot - RLU for negative control of HBsAg test)}} \times 100$$

RLU = relative light units.

14. INTERPRETATION OF RESULTS

The test is valid if neutralization of the LIAISON® XL MUREX HBsAg positive control (REF 317251) is at least 50%.

A specimen is not confirmed positive when the value of the non-neutralized aliquot (mixed with specimen diluent) is less than 0.9 S/CO irrespective of the outcome of percent neutralization (= negative specimen).

A specimen is not confirmed positive when the value of the non-neutralized aliquot (mixed with specimen diluent) is greater than or equal to 0.9 S/CO and percent neutralization is less than 50% (= presence of an interfering substance).

A specimen is confirmed positive when the value of the non-neutralized aliquot (mixed with specimen diluent) is greater than or equal to 0.9 S/CO and percent neutralization is greater than or equal to 50%.

Specimen dilution	S/CO (non-neutralized aliquot)	% Neutralization	Interpretation of results
Neat	< 0.9	Any value	Not confirmed (HBsAg-negative specimen).
Neat	≥ 0.9	< 50%	Not confirmed (interfering substance).
Neat	≥ 0.9	≥ 50%	Confirmed (true HBsAg-positive specimen).

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical sensitivity

Analytical sensitivity was assessed by testing serial dilutions of the Third International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2) NIBSC code: 12/226. Each dilution was treated as indicated in LIAISON® XL MUREX HBsAg Confirmatory instructions for use. When testing with LIAISON® XL MUREX HBsAg, all dilutions were correctly neutralized.

Analytical sensitivity was then calculated by interpolating the signal of the non-neutralized aliquots from the above dilution curve around the point corresponding to the cut-off value, and was found to be less than 0.130 IU/mL, as required by Common Technical Specification 2009/886/EC.

15.2. Diagnostic specificity and sensitivity

Diagnostic specificity was assessed by testing two specimens giving repeatedly reactive results by LIAISON® XL MUREX HBsAg test (REF 317250), but known to be a false positive sample. HBsAg confirmatory test correctly did not confirm those specimens as HBsAg positive.

Diagnostic sensitivity was assessed by testing 422 specimens giving repeatedly reactive results by LIAISON® XL MUREX HBsAg test (REF 317250) and known to be true positive samples. HBsAg Confirmatory Test correctly confirmed those specimens as positive (diagnostic sensitivity: 100% 422/422 – 95% confidence interval: 98.9-100%).

Referring to specimens below 50 S/CO on LIAISON® XL MUREX HBsAg (REF 317250), the diagnostic sensitivity was assessed by testing 33 specimens giving repeatedly reactive results by LIAISON® XL MUREX HBsAg test (REF 317250) and known to be true positive samples. HBsAg confirmatory test correctly confirmed those specimens as positive (diagnostic sensitivity: 100% - 95% confidence interval: 89.6-100%).

In an additional study the ability of the LIAISON® XL MUREX HBsAg Confirmatory assay to neutralize HBsAg positive specimens was evaluated by testing sequentially-collected specimens belonging to 15 seroconversion panels from donors who seroconverted over the course of their donation history. HBsAg confirmatory test correctly confirmed those specimens as positive.

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.

EC REP
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REFERENCES

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2. JV. Parry et al., One or two serological assay testing strategy for diagnosis of HBV and HCV infection? The use of predictive modelling. BMC Infect Dis. 2017 Nov 1.
3. L. Sommese., Comparison between screening and confirmatory serological assays in blood donors in a region of South Italy. J Clin Lab Anal. 2014 May.

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