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The symbols glossary is provided electronically and can be found in the Dialog section at www.diasorin.com using the part and lot numbers associated with the corresponding IVD product.

LIAISON® XL murex HBsAg Qual ([REF] 318250)

1. INTENDED USE

The LIAISON® XL murex HBsAg Qual assay is an *in vitro* chemiluminescent immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric (2 to 21 years) serum and plasma (lithium and sodium heparin, sodium citrate and potassium EDTA) including separator tubes, on the LIAISON® XL Analyzer. Assay results in conjunction with other hepatitis B virus (HBV) serological and clinical information, may be used as an aid in the diagnosis of HBV infection in patients with symptoms of hepatitis or who may be at risk for HBV infection. The assay may also be used to screen for HBV infection in pregnant women to identify neonates who are at risk for acquiring hepatitis B during the perinatal period.

This assay is not approved for use in screening blood, plasma or tissue donors.

Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B is a liver infection caused by the hepatitis B virus (HBV). HBV is an enveloped deoxyribose nucleic acid (DNA) virus.

The complete hepatitis B virus, also called the Dane particle, is composed of an outer surface or envelope that carries the hepatitis B surface antigen (HBsAg). The envelope surrounds an inner core that contains the hepatitis B core antigen (HBcAg). Inside the core is the HBV deoxyribonucleic acid (DNA) genome. Another antigen, the hepatitis B e antigen (HBeAg), is a viral core protein found in the bloodstream during active replication of HBV. Following an infection with HBV, this antigen can be recognized by the infected person's immune system, which will produce antibodies targeting this antigen. The detection of these antibodies against these antigens, form the basis of some serological tests used for diagnosis.

HBV is spread when an uninfected person comes into contact with infected blood or body fluids. Most infections occur via contact with infected blood, but semen, saliva and cervical secretions can also be infectious and Mother-to-Child Transmission (MTCT) of HBV remains an important source of incident cases of HBV. The virus can live on surfaces for at least seven days which means it can also be transmitted via objects that have been contaminated with infected body fluids (e.g. used needles) [1].

HBV is a non-cytopathic virus that causes liver damage through immunopathogenesis [2]. Common symptoms of HBV infection include malaise, fever, gastroenteritis and jaundice. However, some patients may have chronic HBV infection and be asymptomatic. Most infected adults (>90%) will completely recover from an HBV infection, only experiencing a mild, asymptomatic acute illness. In contrast, ~90% of infants and young children will develop a more severe chronic infection [2; 3]. Chronic HBV infection is characterized by the persistence of HBsAg (Hepatitis B surface Antigen) for at least six months and can lead to serious complications such as cirrhosis, hepatocellular carcinoma and liver related mortality. HBV is endemic throughout the world, despite the availability of an efficient vaccine [4, 5, 6].

The detection of HBsAg in human serum or plasma indicates an infection with the Hepatitis B virus (HBV). In acute hepatitis B infection the first immunological marker to appear is HBsAg which may be present some days or weeks before clinical symptoms begin to appear. HBsAg is found in patients with both acute and chronic hepatitis B infections.[7]

Diagnosis of HBV infection is achieved through blood or serum detection of viral nucleic acid or serological markers (i.e. proteins produced by the virus or antibodies produced by the host that target viral proteins). Serological tests are commonly used to detect HBV infection status and are the first testing strategy to screen for HBV exposure. Common HBV serological markers include HBsAg, antibody to HBsAg (anti-HBs), total antibody to HBcAg (total anti-HBc), immunoglobulin M (IgM) antibody to HBcAg (IgM anti-HBc), HBeAg and antibody to HBeAg (anti-HBe). The detection of these serological markers allows identification of past or ongoing HBV infection, assessment of the clinical phases of the disease, monitoring of antiviral therapy and determination of the immune status of the patient [8-12].

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of HBsAg is a direct sandwich chemiluminescence immunoassay (CLIA). 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 second 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 coated with antibodies to HBsAg (mouse monoclonal), biotinylated BSA, streptavidin, BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (3.0 mL)	**[CAL|1]**	Low levels of recombinant HBsAg (obtained in mammalian cells), BSA, phosphate buffer, EDTA, 0.2% ProClin® 300, an inert yellow dye.
Buffer L (28 mL)	**[BUF|L]**	Non-specific IgG (mouse polyclonal), casein, TRIS buffer, EDTA, 0.1% ProClin® 300.
Conjugate (2 x 23 mL)	**[CONJ]**	Mouse Monoclonal antibodies to HBsAg, conjugated to an isoluminol derivate, human and animal sera, BSA, phosphate buffer, preservatives.
Number of tests		200

ProClin® is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

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)

LIAISON® XL Starter Kit ([REF] 319200)

LIAISON® Wash/System Liquid ([REF] 319100)

LIAISON® XL Waste Bags ([REF] X0025)

Additionally required materials

LIAISON® XL MUREX HBsAg Qual controls (negative and positive) ([REF] 318251)

LIAISON® XL MUREX HBsAg Confirmatory ([REF] 318110)

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For Prescription Use Only
- All human blood source material used to produce the components provided are derived from units found to be non-reactive for HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 when tested by an FDA-approved method. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen in the CDCNIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 2007, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections.
- Observe the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette by mouth.
- Strict adherence to the instructions is necessary to obtain reliable results.
- Avoid direct contact with potentially infected material by wearing laboratory coats, 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 10% sodium hypochlorite solution (containing 0.5% active chlorine), and the means used must be treated as infected waste.
- All samples, biological reagents and disposable materials used in the assay must be considered as potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory and the regulations of each Country.
- Liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 10% for at least half an hour.
- Any materials to be reused must be appropriately sterilized in compliance with the local laws and guidelines. A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- The LIAISON® Analyzer family should be cleaned and decontaminated on a routine basis. See the relevant Operator's Manual for the procedures.
- Do not use kits or components beyond the expiration date given on the label.
- Do not mix reagents from different reagents packs (even for the same reagent).
- Previously frozen samples should be thoroughly mixed after thawing and prior to testing.
- A non-reactive test result for HBsAg does not exclude the possibility of exposure to or infection with HBV.
- 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 HBsAg in the population being screened.
- 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

Chemical Hazard and Safety Information

- Reagents in this kit are classified in accordance with the US OSHA Hazard Communication Standard; individual US State Right-to-Know laws and European Union EC Regulation 1272/2008 (CLP).
- Hazardous reagents are classified and labelled as follows:

REAGENTS:	[CAL|1], [CONJ],	[BUF|L]
CLASSIFICATION:	Skin sens. 1 H317	Skin sens. 1 H317 Acute Tox. 3 H331 Eye Dam. 1 H318 Skin irrit. 2 H315 Aquatic Chronic Tox. 3 H412
SIGNAL WORD:	Warning	Danger
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	 GHS05 Corrosion, GHS06 Skull and crossbones
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction.	H331 Toxic if inhaled H318 Causes serious eye damage H315 Causes skin irritation 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	P261 Avoid breathing dust/fume/gas/mist/vaporous/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/doctor/… 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).	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).	N-Lauroylsarcosine salt 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) Triton X-100

Reagents containing sodium azide

Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

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.

6. PREPARATION OF 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 resuspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the color of the suspension has changed to brown.
- Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation).
- Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have been resuspended.

- Carefully wipe the surface of each septum to remove residual liquid.
- Repeat as necessary until the magnetic particles are completely resuspended.

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 to ensure there is no foaming present before using the Integral. If foam is present after resuspension of the magnetic particles, place the Integral on the instrument and allow the foam to dissipate.

The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area
– LIAISON® 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 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.

7. STORAGE AND STABILITY OF REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles. See the Preparation of Reagent Integral section for resuspension instructions. When the Reagent Integral is stored sealed and kept upright, the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral should not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing the seals, the Reagent Integral is stable for twelve (12) weeks when stored at 2-8°C in a refrigerator or on board the analyzer.

8. SPECIMEN COLLECTION AND PREPARATION

Either human serum, serum in serum-separating-tube (SST) or plasma may be used. The results obtained on the serum-plasma paired samples indicated that there is equivalence among serum (with and without gel SST), K_2 EDTA, Lithium Heparin, Sodium Heparin and Sodium Citrate.
Blood should be collected aseptically by venipuncture, allowed to clot (if applicable), and the serum or plasma should be separated from the red cells as soon as possible. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested.
Check for and remove air bubbles and foam before assaying. A limited time of room temperature storage (between 18 and 30°C) for three (3) days does not influence the assay performance. If the assay is performed within seven (7) days of sample collection, the samples may be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Samples are stable through seven (7) freeze/thaw cycles. Self-defrosting freezers are not recommended for sample storage.
It is responsibility of the individual laboratory to use all available references and/or its own studies to determinate specific stability criteria for its laboratory.

The minimum specimen volume required for a single determination is 300 µL (150 µL specimen + 150 µL dead volume). Dead volume is the volume left at the bottom of the aliquot tube which the instrument cannot aspirate.

Additional volume is required for samples that are repeatedly reactive and require confirmatory testing. Refer to the LIAISON® XL MUREX HBsAg Confirmatory assay ([REF] 318110) Instructions for Use-section 8

For shipping, use sterile containers and pack specimens in compliance with government regulations covering the transportation of etiologic agents. Ensure that specimens reach their destination within the following specifications:
- Plasma and Serum separated from the clot can be maintained at 2-8°C during transit. Do not exceed the maximum 2-8°C stability of seven (7) days.
- Plasma and Serum separated from the clot can be stored at -20°C or below and shipped with dry ice. Temperature level during entire shipment should be no greater (warmer) than -20°C.

9. 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 into the reaction cuvettes.
2. Dispense coated magnetic particles into the reaction cuvettes.
3. Dispense Buffer L into the reaction cuvettes.
4. Incubate
5. Dispense conjugate into the reaction cuvettes.
6. Incubate.
7. Wash with Wash/System liquid.
8. Add the Starter Reagents and measure the light emitted.

Due to the presence of detergents in the LIAISON® XL MUREX HBsAg Quant 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.

10. CALIBRATION

Assaying of the calibrator contained in the reagent integral allows the analyzer to set the assay cut-off. The 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 reagent integral or Starter Kit is used.
- The previous calibration was performed more than eight (8) weeks before.
- The analyzer has been serviced.
- Control values lie outside the expected ranges

Calibrator values are stored in the Radio Frequency Identification transponder (RFID Tag).

11. QUALITY CONTROL

Quality control must be performed once per day of use or in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. It is recommended that the user refer to CLSI document, C24-A3, and 42 CFR 493.1256(c) for guidance on appropriate quality control practices.
LIAISON® controls should be run in singlicate to monitor the assay performance.
If control values lie within the expected ranges, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and specimens must be retested.

12. 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.
- Do not heat-inactivate sera.
- This test is suitable only for investigating single samples, not for diluted specimens, sample pools or heat-inactivated specimens.
- If the LIAISON® XL MUREX HBsAg Qual results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

13. INTERPRETATION OF RESULTS

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

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

- Cut-off value of a 1.00 S/CO determines whether a sample has detectable levels of HBsAg.
- **Reactive:** Samples with HBsAg levels equal to or above a S/CO value of 1.00 are considered initially Reactive and presumed positive for HBsAg.
- **Non-Reactive:** Samples with HBsAg levels below a S/CO value of 1.00 are considered Non-reactive and presumed negative for HBsAg.

Initially Reactive samples must be retested in duplicate. Samples with HBsAg levels below a 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 must be evaluated with the LIAISON® XL MUREX HBsAg Confirmatory Test [REF] 318110.

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1 Summary of clinical performance

A multi-site clinical agreement study was conducted to determine the clinical performance of the LIAISON® XL MUREX HBsAg Qual assay. The tested clinical study population was made up of a total of 3882 specimens consisting of:

- a total of 2826 prospective specimens, collected unselected;
- a total of 256 selected retrospective specimens;
- a total of 800 prospective specimens collected from pregnant subjects during the first, second and third trimester.

14.1 Summary of clinical performance

The LIAISON® XL MUREX HBsAg Qual clinical study population consisted of a total of 3082 specimens of which 2826 were collected prospectively from individuals at increased risk of HBV infection due to lifestyle, behavior, occupation, disease state or a known exposure event, or from individuals with signs and symptoms of hepatitis infection (asymptomatic and symptomatic). A demographic summary of the overall risk specimen population by gender and race is provided in the following tables:

Demographics of Clinical Study Samples by Gender

	Adult				Pediatric (2-21)				Unknown Age			
	Prospective		Retrospective		Prospective		Retrospective		Prospective		Retrospective	
Gender	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Female	1643	61.7%	54	26.2%	98	60.9%	2	6.5%	1	50.0%	0	0.0%
Male	1017	38.2%	151	73.3%	61	37.9%	29	93.5%	1	50.0%	0	0.0%
Unknown	3	0.1%	1	0.5%	2	1.2%	0	0.0%	0	0.0%	21	100.0%
Total	**2663**	**100.0%**	**206**	**100.0%**	**161**	**100.0%**	**31**	**100.0%**	**2**	**100.0%**	**21**	**100.0%**

Demographics of Clinical Study Samples by Race

	Adult				Pediatric (2-21)				Unknown Age			
	Prospective		Retrospective		Prospective		Retrospective		Prospective		Retrospective	
Race	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
American Indian/ Alaskan Native	2	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Asian	21	0.8%	4	1.9%	3	1.9%	0	0.0%	0	0.0%	0	0.0%
Black/ African American	832	31.2%	57	27.7%	64	39.8%	4	13.8%	0	0.0%	0	0.0%
Native Hawaiian or Other Pacific Islander	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
White	1664	62.5%	141	68.4%	89	55.3%	27	86.2%	2	100.0%	21	100.0%
Unknown	6	0.2%	1	0.5%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Other	138	5.2%	3	1.5%	5	3.1%	0	0.0%	0	0.0%	0	0.0%
Total	**2663**	**100.0%**	**206**	**100.0%**	**161**	**100.0%**	**31**	**100.0%**	**2**	**100.0%**	**21**	**100.0%**

HBV serological classification for prospective and retrospective specimens is presented in the next table. Classification was based on results of the complete hepatitis B panel using FDA approved assays to determine the disease state. For serological characterization, the following HBV markers were evaluated: HBsAg (and HBsAg Confirmatory test), Anti-HBs, Anti-HBc IgM, Anti-HBc, Anti-HBe and HBeAg.

Serological Classification by FDA-Approved HBV Panel

HBV Classification	HBsAg	HBeAg	Anti-HBc	Anti-HBc IgM	Anti-HBe	Anti-HBs	Prospective (n)	Retrospective (n)
Acute	R	NR	NR	NR	NR	NR	12	97
Acute	R	R	NR	NR	NR	NR		
Acute	R	R	R	R	NR	NR		
Acute	R	R	R	R	R	NR		
Acute	R	R	R	R	EQV	NR		
Acute	R	NR	R	EQV	R	NR		
Acute	R	NR	R	R	EQV	NR		
Acute	R	EQV	R	R	R	NR		
Acute	R	NR	R	R	NR	NR		
Acute	R	R	R	EQV	NR	NR		
Acute	R	R	R	R	NR	R		
Acute	R	R	R	R	EQV	R		
Acute	R	R	R	R	R	EQV		
Late Acute	R	NR	R	R	R	NR	2	32
Late Acute	R	NR	R	R	R	R		
Chronic	R	NR	NR	NR	R	NR	76	68
Chronic	R	NR	R	NR	NR	R		
Chronic	R	R	R	NR	NR	R		
Chronic	R	R	R	NR	NR	NR		

HBV Classification	HBsAg	HBeAg	Anti-HBc	Anti-HBc IgM	Anti-HBe	Anti-HBs	Prospective (n)	Retrospective (n)
Chronic	R	EQV	R	NR	NR	NR		
Chronic	R	NR	R	NR	R	NR		
Chronic	R	NR	R	NR	NR	NR		
Chronic	R	NR	R	NR	R	R		
Chronic	R	EQV	R	NR	NR	NR		
Early Recovery	NR	NR	R	R	R	NR	48	9
Early Recovery	NR	NR	R	EQV	R	R		
Early Recovery	NR	NR	R	R	NR	NR		
Early Recovery	NR	NR	R	NR	R	NR		
Early Recovery	NR	NR	R	NR	NR	NR		
Early Recovery	NR	NR	R	R	NR	R		
Early Recovery	NR	NR	R	R	R	R		
Recovery	NR	NR	R	NR	R	R	131	36
Recovery	NR	NR	NR	NR	R	R		
Recovery	NR	NR	R	NR	EQV	R		
Immune Due to Natural Infection	NR	NR	R	NR	NR	R	104	3
Immune Due to Natural Infection	NR	NR	R	NR	NR	EQV		
HBV Vaccine Response	NR	NR	NR	NR	NR	R	1144	8
HBV Vaccine Response	NR	NR	NR	NR	NR	EQV		
Not Previously Infected	NR	NR	NR	NR	NR	NR	1302	1
Not Interpretable	NR	NR	NR	NR	R	NR	7	2
Not Interpretable	NR	NR	NR	R	NR	NR		
Not Interpretable	NR	R	NR	NR	NR	NR		
Not Interpretable	NR	R	NR	NR	NR	R		
Not Interpretable	NR	R	R	R	NR	EQV		
Not Interpretable	NR	R	R	R	NR	R		
Not Interpretable	R	NR	NR	NR	NR	R		
Total							2826	256

Based on the HBV classifications, the LIAISON® XL MUREX HBsAg Qual results for the 2826 prospective and 256 retrospective specimens were compared to a reference HBsAg assay. Samples found repeatedly reactive for HBsAg by LIAISON® XL MUREX HBsAg Qual were confirmed for the presence of HBsAg with the LIAISON® XL MUREX HBsAg Confirmatory. The following tables show this comparison and percent agreement with 95% confidence intervals with the reference HBsAg assay results.

Cumulative Adult and Pediatric Clinical Agreement (Combined Prospective & Retrospective*)
LIAISON® XL MUREX HBsAg Qual vs Reference Assay by Characterization

HBV Classification	Reference HBsAg assay				Total
	Reactive		Non reactive		
	LIAISON® XL MUREX HBsAg Qual		LIAISON® XL MUREX HBsAg Qual		
	Reactive	Non reactive	Reactive	Non reactive	
Acute	106	3	0	0	109
Late Acute	33	1	0	0	34
Chronic	144	0	0	0	144
Early Recovery	0	0	0	57	57
Recovery	0	0	4	163	167
Immune Due to Natural Infection	0	0	0	107	107
HBV Vaccine Response	0	0	0	1152	1152
Not Previously Infected	0	0	2	1301	1303
Not Interpretable	0	1	0	8	9
Total	**283**	**5**	**6**	**2788**	**3082**

Cumulative Adult and Pediatric Clinical Agreement Summary (Combined Prospective & Retrospective)

LIAISON® XL MUREX HBsAg vs Reference Assay by Characterization

HBV Classification	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
Acute	106/109 (97.2%) 95% CI: 92.2% to 99.1%	N/A
Late Acute	33/34 (97.1%) 95% CI: 85.1% to 99.5%	N/A
Chronic	144/144 (100.0%) 95% CI: 97.4% to 100.0%	N/A
Early Recovery	N/A	57/57 (100.0%) 95% CI: 93.7% to 100.0%
Recovery	N/A	163/167 (97.6%) 95% CI: 94.0% to 99.1%
Immune Due to Natural Infection	N/A	107/107 (100.0%) 95% CI: 96.5% to 100.0%
HBV Vaccine Response	N/A	1152/1152 (100.0%) 95% CI: 99.7% to 100.0%
Not Previously Infected	N/A	1301/1303 (99.8%) 95% CI: 99.4% to 100.0%
Not Interpretable	0/1 (0.0%) 95% CI: 0.0% to 79.3%	8/8 (100.0%) 95% CI: 67.6% to 100.0%
Total	**283/288 (98.3%) 95% CI: 96.0% to 99.3%**	**2788/2794 (99.8%) 95% CI: 99.5% to 99.9%**

The LIAISON® XL MUREX HBsAg Qual results for the 800 prospective specimens collected from pregnant subjects at first, second and third trimester were compared to a reference HBsAg assay. The following tables show this comparison and percent agreement with 95% exact confidence intervals with the reference HBsAg assay results.

Clinical Agreement Pregnancy Prospective Collection
LIAISON® XL MUREX HBsAg Qual vs Reference Assay by Trimester

Pregnancy by Trimester	Reference HBsAg assay				Total
	Reactive		Non reactive		
	LIAISON® XL MUREX HBsAg Qual		LIAISON® XL MUREX HBsAg Qual		
	Reactive	Non reactive	Reactive	Non reactive	
First	2	0	0	380	382
Second	0	0	0	193	193
Third	1	0	0	189	190
Unknown	1	0	0	34	35
Total	**4**	**0**	**0**	**796**	**800**

Clinical Agreement Pregnancy
LIAISON® XL MUREX HBsAg vs Reference Assay by Trimester

Pregnancy by Trimester	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
First	2/2 (100.0%) 95% CI: 34.2% to 100.0%	380/380 (100.0%) 95% CI: 99.0% to 100.0%
Second	N/A	193/193 (100.0%) 95% CI: 98.0% to 100.0%
Third	1/1 (100.0%) 95% CI: 20.7% to 100.0%	189/189 (100.0%) 95% CI: 98.0% to 100.0%
Unknown	1/1 (100.0%) 95% CI: 20.7% to 100.0%	34/34 (100.0%) 95% CI: 89.8% to 100.0%
Total	**4/4 (100.0%) 95% CI: 51.0% to 100.0%**	**796/796 (100.0%) 95% CI: 99.5% to 100.0%**

Cumulative Pediatric Clinical Agreement Summary (Combined Prospective & Retrospective)
LIAISON® XL MUREX HBsAg vs Reference Assay by Characterization.

HBV Classification	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
Acute	20/20 (100.0%) 95% CI: 83.9% to 100.0%	N/A
Late Acute	7/7 (100.0%) 95% CI: 64.6% to 100.0%	N/A
Chronic	7/7 (100.0%) 95% CI: 64.6% to 100.0%	N/A
Early Recovery	N/A	1/1 (100.0%) 95% CI: 20.7% to 100.0%
Recovery	N/A	5/5 (100.0%) 95% CI: 56.6% to 100.0%
Immune Due to Natural Infection	N/A	3/3 (100.0%) 95% CI: 43.9% to 100.0%N/A
HBV Vaccine Response	N/A	63/63 (100.0%) 95% CI: 94.3% to 100.0%
Not Previously Infected	N/A	84/84 (100.0%) 95% CI: 95.6% to 100.0%
Not Interpretable	N/A	2/2 (100.0%) 95% CI: 34.2% to 100.0%
Total	34/34 (100%) 95% CI:89.5% to 100.0%	158/158 (100%) 95% CI: 97.6% to 100.0%

14.2 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 suggest that pediatric samples react in the same manner as adult samples.

14.3. Precision with LIAISON® XL Analyzer

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 Qual kit lots in two (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 Qual 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
Kit Ctrl Neg lot 1	240	0.41	0.023	5.8%	0.023	5.6%	0.026	6.5%	0.035	8.6%	0.055	13.5%
Kit Ctrl Neg lot 2	240	0.41	0.026	6.4%	0.016	4.0%	0.029	7.2%	0.031	7.5%	0.053	12.8%
Kit Ctrl Neg lot 3	240	0.41	0.026	6.2%	0.014	3.4%	0.031	7.5%	0.035	8.4%	0.055	13.3%
Kit Ctrl Pos lot 1	240	1.96	0.070	3.6%	0.041	2.1%	0.085	4.4%	0.096	4.9%	0.152	7.8%
Kit Ctrl Pos lot 2	240	1.83	0.062	3.4%	0.036	2.0%	0.082	4.5%	0.065	3.5%	0.127	7.0%
Kit Ctrl Pos lot 3	240	1.79	0.059	3.3%	0.034	1.9%	0.081	4.5%	0.060	3.4%	0.122	6.8%
HBS1U10	240	0.39	0.030	7.9%	0.034	8.9%	0.022	5.8%	0.017	4.3%	0.054	13.9%
HBS1U11	240	0.73	0.035	4.8%	0.021	2.9%	0.039	5.3%	0.025	3.4%	0.062	8.4%
HBS1U12	240	0.72	0.030	4.1%	0.019	2.6%	0.046	6.3%	0.027	3.7%	0.064	8.8%
HBS1U13	240	0.72	0.028	3.9%	0.021	2.9%	0.039	5.5%	0.028	3.9%	0.060	8.3%
HBS1U14	240	1.12	0.037	3.3%	0.024	2.1%	0.082	7.3%	0.045	4.0%	0.103	9.2%
HBS1U15	240	1.12	0.039	3.5%	0.034	3.1%	0.082	7.3%	0.044	3.9%	0.106	9.5%
HBS1U16	240	1.13	0.039	3.4%	0.034	3.0%	0.074	6.5%	0.041	3.7%	0.099	8.8%
HBS1U17	240	3.02	0.079	2.6%	0.078	2.6%	0.114	3.8%	0.129	4.3%	0.205	6.8%
HBS1U18	240	3.06	0.057	1.8%	0.057	1.9%	0.135	4.4%	0.128	4.2%	0.202	6.6%
HBS1U19	240	3.04	0.055	1.8%	0.063	2.1%	0.122	4.0%	0.108	3.5%	0.183	6.0%
HBS1U20	240	1.06	0.035	3.3%	0.020	1.8%	0.042	4.0%	0.037	3.5%	0.069	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 Qual 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.

Sample ID	N	LIAISON® XL MUREX HBsAg Qual Assay 5 Day Multi-Site / Multi-Lot										
		Mean	Repeatability		Between-Day/Runs		Within Laboratory		Between Sites/Lots		Reproducibility	
		(S/CO)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Neg (all 3 lots)	90	0.38	0.033	8.6%	0.029	7.7%	0.044	11.5%	0.044	11.6%	0.062	16.3%
Ctrl Pos (all 3 lots)	90	1.859	0.041	2.2%	0.04	2.2%	0.058	3.1%	0.052	2.8%	0.077	4.2%
HBS1U10	90	0.342	0.022	6.5%	0.023	6.8%	0.032	9.4%	0.023	6.8%	0.04	11.6%
HBS1U11	90	0.734	0.026	3.5%	0.031	4.2%	0.04	5.4%	0.042	5.8%	0.058	7.9%
HBS1U12	90	0.726	0.029	4.0%	0.03	4.1%	0.042	5.8%	0.043	5.9%	0.06	8.2%
HBS1U13	90	0.721	0.031	4.3%	0.033	4.6%	0.045	6.3%	0.03	4.1%	0.054	7.5%
HBS1U14	90	1.138	0.033	2.9%	0.03	2.7%	0.045	3.9%	0.038	3.4%	0.059	5.2%
HBS1U15	90	1.135	0.031	2.7%	0.041	3.6%	0.051	4.5%	0.036	3.2%	0.062	5.5%
HBS1U16	90	1.146	0.035	3.0%	0.037	3.2%	0.051	4.4%	0.026	2.3%	0.057	5.0%
HBS1U17	90	3.213	0.065	2.0%	0.051	1.6%	0.083	2.6%	0.116	3.6%	0.143	4.4%
HBS1U18	90	3.196	0.057	1.8%	0.04	1.2%	0.069	2.2%	0.104	3.2%	0.125	3.9%
HBS1U19	90	3.211	0.066	2.1%	0.052	1.6%	0.084	2.6%	0.107	3.3%	0.136	4.2%
HBS1U20	90	1.099	0.034	3.1%	0.034	3.1%	0.048	4.3%	0.05	4.5%	0.069	6.3%

14.4 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 both with LIAISON® XL MUREX HBsAg Qual and a FDA-approved reference assay.

14.5 HBsAg mutant detection

A panel of ten (10) recombinant mutants were tested with the LIAISON® XL MUREX HBsAg Qual and a FDA-approved reference 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 Qual.

Sample ID	Mutation	Comparator HBsAg assay	LIAISON® XL MUREX HBsAg Qual 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

14.6 Analytical sensitivity

In order to determine sensitivity of LIAISON® XL MUREX HBsAg Qual 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 Qual assay at cutoff level is 0.05 IU/mL.

14.7. Analytical Sensitivity as Seroconversion Panel Performance

Thirty (30) commercially available HBsAg seroconversion panels were tested using LIAISON® XL MUREX HBsAg Qual and a commercially available FDA-approved HBsAg comparator assay to determine the sensitivity of the assay. The results are summarized in the following table:

		LIAISON XL MUREX HBSAg Qual		Comparator Assay		Difference in number of days	Difference in number of blood Draws
Panel ID	Number of samples	Last Bleed (Day) with Non-Reactive Result	First Bleed (Day) with Reactive result	Last Bleed (Day) with Non-Reactive Result	First Bleed (Day) with Reactive result		
PHM 937	5	2	9	2	9	0	0
PHM941	5	7	14	7	14	0	0
HBV11000	9	21	26	21	26	0	0
HBV11001	8	35	44	35	44	0	0
HBV11002	5	9	35	7	9	26	1
HBV11003	6	135	142	135	142	0	0
HBV11004	7	15	48	15	48	0	0
HBV11005	14	112	142	112	142	0	0
HBV11006	17	44	49	44	49	0	0
HBV11007	11	36	41	36	41	0	0
HBV11008	18	72	79	69	72	7	1
HBV11011	14	105	110	105	110	0	0
HBV11012	6	18	20	18	20	0	0
HBV11013	15	246	251	246	251	0	0
HBV11014	10	37	51	37	51	0	0
HBV11015	9	41	43	41	43	0	0
HBV11016	9	27	30	27	30	0	0
HBV11017	14	42	47	42	47	0	0
HBV11028	10	30	35	30	35	0	0
HBV6273	6	14	25	14	25	0	0
HBV6274	7	0	4	0	4	0	0
HBV6275	7	9	22	9	22	0	0
HBV6276	8	27	29	27	29	0	0
HBV6277	11	35	40	35	40	0	0
HBV6278	10	8	12	8	12	0	0
HBV6290	12	21	23	21	23	0	0
HBV11009	23	81	86	81	86	0	0
HBV9074	20	70	73	70	73	0	0
PHM926	8	13	15	13	15	0	0
PHM939	5	3	11	14	21	-10	-2

In all panels the LIAISON® XL MUREX HBsAg Qual assay shows detection of seroconversion equal to the reference HBsAg assay except with three panels. The LIAISON® XL MUREX HBsAg Qual assay detected seroconversion to a reactive status one draw later than the reference assay in panel HBV11002 and HBV11008, while it detected two draws earlier than the reference assay in panel PHM939.

14.8. Matrix Comparison

Twenty-five (25) paired sets of matched serum (with and without gel SST) and plasma (lithium and sodium heparin, sodium citrate and K_2 EDTA) were tested to determine if these sample types provide equivalent results on the LIAISON® XL MUREX HBsAg Qual assay. Each sample was divided into three aliquots. Two sets of aliquots were spiked with an HBsAg high positive sample to achieve two (2) levels of samples: high negative and low positive samples. The third set of aliquots was un-spiked to serve as control samples. Where possible, native samples identified as high negative and low positive during the initial screening, were used instead of spiking these samples. The results of the negative and low positive samples did not change the classification of the expected result. The results obtained on the serum-plasma paired samples indicated that there is equivalence among serum (with and without gel SST), K_2 EDTA, lithium heparin, sodium citrate and sodium heparin plasma.

14.9 Potential interfering substances

Controlled studies of potentially interfering substances at two (2) HBsAg levels around the cutoff, showed no interference at the concentration for each substance listed below in the LIAISON® XL MUREX HBsAg Qual assay. The testing was based on CLSI-EP07.

Substances	Tested concentrations
Triglycerides	3000 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Albumin	6000 mg/dL
Cholesterol	400 mg/dL
Vitamin H (Biotin)	3500 ng/mL

14.10 Cross-reactivity

The LIAISON® XL MUREX HBsAg Qual 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 samples were found to be non-reactive with both the LIAISON® XL MUREX HBsAg Qual assay and the FDA-approved HBsAg reference assay while one (1) sample was found to be non-reactive with the reference assay and reactive with the LIAISON® XL MUREX HBsAg Qual assay. Conclusion: No potential interference is demonstrated by the medical conditions presented in this comparison study.

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

In addition, a study was conducted to evaluate the LIAISON® XL MUREX HBsAg Qual assay for potential cross-reactivity in specimens from individuals with various medical conditions (active infection as demonstrated by the presence of the antigen/confirmed by NA or PCR/IgM positive). The specimens were evaluated with the LIAISON® XL MUREX HBsAg Qual assay and a reference assay. The results are summarized in the following table.

Potential cross reactant	N	Comparator HBsAg Assay	LIAISON XL MUREX HBsAg Qual	
			Non reactive	Reactive
Syphilis (*T. Pallidum*)	10	Negative	10	0
Toxoplasmosis (*Toxoplasma gondii*)	10	Negative	10	0
CMV (Cytomegalovirus)	13	Negative	13	0
EBV (Epstein-Barr virus)	10	Negative	10	0
HAV (Hepatitis A virus)	10	Negative	10	0
HIV (human immunodeficiency virus)	27	Negative	26	1
HSV (herpes simplex virus)	10	Negative	10	0
HTLV (Human T-lymphotropic virus)-1/2	7	Negative	7	0
Parvovirus B19	10	Negative	10	0
Rubella virus	10	Negative	9	1
Varicella-zoster virus	12	Negative	10	2
N.gonorrhoeae	12	Negative	12	0
T.Cruzi	10	Negative	10	0
Staphylococcus aureus	10	Negative	10	0
Pseudomonas auruginosa	10	Negative	10	0
E.Coli	10	Negative	10	0
Hepatitis C (HCV)	10	Negative	10	0
Chlamydia (*C.Trachomatis*)	15	Negative	14	1

In conclusion 201 samples were found to be non-reactive with both the LIAISON® XL MUREX HBsAg Qual assay and the FDA-approved HBsAg reference assay. Only 1 sample was found to be non-reactive with the reference assay and reactive with the LIAISON® XL MUREX HBsAg Qual.

Five (5) samples tested resulted Reactive with the LIAISON® XL MUREX HBsAg Qual assay:
- 1 sample for Chlamydia: Confirmatory testing could not be performed on the sample as the sample volume was depleted, therefore the reactivity could not be confirmed
- 1 sample for HIV and 2 samples for Varicella-zoster virus were non-reactive by confirmatory testing, therefore these samples are considered true cross-reactants
- 1 sample for Rubella was graded as reactive by confirmatory testing, this sample is considered a true HBsAg reactive sample.

The LIAISON® XL MUREX HBsAg Qual assay demonstrated suitable specificity performance for each of the potentially cross-reactant pathologies as results were comparable to the results of the reference test method.

Potential interference from bacterial and viral proteins was evaluated by testing an HBsAg-reactive panel as well as an HBsAg-non-reactive panel with the LIAISON® XL MUREX HBsAg Qual assay. Each panel contained individual samples spiked with specific bacterial or viral culture materials at two concentrations (1000 and 10000 cfu/mL for bacteria and 1 and 1000 ng/mL for viruses) and an unspiked control.
These samples were only tested in the LIAISON® XL MUREX HBsAg Qual assay. The unspiked control results were compared to the results obtained with the spiked materials.
The results of bacterial spikes and viral antigen spikes are provided in the following tables:

HBsAg results of various bacterial spikes

Samples	Potential Interferant Concentration	LIAISON® XL MUREX HBsAg Qual assay result	
		before spike	after spike
Unspiked / HBsAg non-reactive panel			
Control	*n.a.*	*Non reactive*	*n.a.*
Spiked / HBsAg non-reactive panel			
Staphylococcus aureus	10^3 CFU/mL	*Non reactive*	*Non reactive*
	10^4 CFU/mL	*Non reactive*	*Non reactive*
Pseudomonas aeruginosa	10^3 CFU/mL	*Non reactive*	*Non reactive*
	10^4 CFU/mL	*Non reactive*	*Non reactive*
E.Coli	10^3 CFU/mL	*Non reactive*	*Non reactive*
	10^4 CFU/mL	*Non reactive*	*Non reactive*
N.gonorrheae	10^3 CFU/mL	*Non reactive*	*Non reactive*
	10^4 CFU/mL	*Non reactive*	*Non reactive*
Chlamydia (*C.Trachomatis*)	10^3 CFU/mL	*Non reactive*	*Non reactive*
	10^4 CFU/mL	*Non reactive*	*Non reactive*
Unspiked / HBsAg reactive panel			
Control	*n.a.*	*Reactive*	*n.a.*
Spiked / HBsAg reactive panel			
Staphylococcus aureus	10^3 CFU/mL	*Reactive*	*Reactive*
	10^4 CFU/mL	*Reactive*	*Reactive*
Pseudomonas Aeruginosa	10^3 CFU/mL	*Reactive*	*Reactive*
	10^4 CFU/mL	*Reactive*	*Reactive*
E.Coli	10^3 CFU/mL	*Reactive*	*Reactive*
	10^4 CFU/mL	*Reactive*	*Reactive*
N.Gonorrheae	10^3 CFU/mL	*Reactive*	*Reactive*
	10^4 CFU/mL	*Reactive*	*Reactive*
Chlamydia (*C.Trachomatis*)	10^3 CFU/mL	*Reactive*	*Reactive*
	10^4 CFU/mL	*Reactive*	*Reactive*

HBsAg results of various viral antigen spikes

Samples	Potential Interferant Concentration	LIAISON® XL MUREX HBsAg Qual assay results	
		before spike	after spike
Unspiked / HBsAg non-reactive panel			
Control	*n.a.*	*Non reactive*	*n.a.*
Spiked / HBsAg non-reactive panel			
Cytomegalovirus	10^3 PFU/mL	*Non reactive*	*Non reactive*
	10^4 PFU/mL	*Non reactive*	*Non reactive*
EBV	10^5 copies/mL	*Non reactive*	*Non reactive*
	10^6 copies/mL	*Non reactive*	*Non reactive*
Varicella-zoster virus	10^5 copies/mL	*Non reactive*	*Non reactive*
	10^6 copies/mL	*Non reactive*	*Non reactive*
Hepatitis A (HAV)	10^5 copies/mL	*Non reactive*	*Non reactive*
	10^6 copies/mL	*Non reactive*	*Non reactive*
HSV-1	10^5 copies/mL	*Non reactive*	*Non reactive*
	10^6 copies/mL	*Non reactive*	*Non reactive*
HIV-1	10^3 U/mL	*Non reactive*	*Non reactive*
	10^4 U/mL	*Non reactive*	*Non reactive*
HIV-2	10^3 U/mL	*Non reactive*	*Non reactive*

Samples	Potential Interferant Concentration	LIAISON® XL MUREX HBsAg Qual assay results	
		before spike	after spike
	10^4 U/mL	*Non reactive*	*Non reactive*
HCV	10^3 IU/mL	*Non reactive*	*Non reactive*
	10^4 IU/mL	*Non reactive*	*Non reactive*
Parvovirus B19	10^3 IU/mL	*Non reactive*	*Non reactive*
	10^4 IU/mL	*Non reactive*	*Non reactive*
Rubella Virus	1 ng/mL	*Non reactive*	*Non reactive*
	1^3 ng/mL	*Non reactive*	*Non reactive*
HTLV-1	1 ng/mL	*Non reactive*	*Non reactive*
	1^3 ng/mL	*Non reactive*	*Non reactive*
Toxoplasmosis	1 ng/mL	*Non reactive*	*Non reactive*
	1^3 ng/mL	*Non reactive*	*Non reactive*
Control	*n.a.*	*Reactive*	*n.a.*
Spiked / HBsAg reactive panel			
Cytomegalovirus	10^3 PFU/mL	*Reactive*	*Reactive*
	10^4 PFU/mL	*Reactive*	*Reactive*
EBV	10^5 copies/mL	*Reactive*	*Reactive*
	10^6 copies/mL	*Reactive*	*Reactive*
Varicella-zoster virus	10^5 copies/mL	*Reactive*	*Reactive*
	10^6 copies/mL	*Reactive*	*Reactive*
Hepatitis A (HAV)	10^5 copies/mL	*Reactive*	*Reactive*
	10^6 copies/mL	*Reactive*	*Reactive*
HSV-1	10^5 copies/mL	*Reactive*	*Reactive*
	10^6 copies/mL	*Reactive*	*Reactive*
HIV-1	10^3 U/mL	*Reactive*	*Reactive*
	10^4 U/mL	*Reactive*	*Reactive*
HIV-2	10^3 U/mL	*Reactive*	*Reactive*
	10^4 U/mL	*Reactive*	*Reactive*
HCV	10^3 IU/mL	*Reactive*	*Reactive*
	10^4 IU/mL	*Reactive*	*Reactive*
Parvovirus B19	10^3 IU/mL	*Reactive*	*Reactive*
	10^4 IU/mL	*Reactive*	*Reactive*
Rubella Virus	1 ng/mL	*Reactive*	*Reactive*
	10^3 ng/mL	*Reactive*	*Reactive*
HTLV-1	1 ng/mL	*Reactive*	*Reactive*
	10^3 ng/mL	*Reactive*	*Reactive*
Toxoplasmosis	1 ng/mL	*Reactive*	*Reactive*
	10^3 ng/mL	*Reactive*	*Reactive*

The bacterial/viral spike performance demonstrated that the LIAISON® XL MUREX HBsAg Qual assay was not affected by the two levels of bacterial and viral proteins introduced through cultured material. The bacterial and viral spiked samples in the HBsAg non-reactive and reactive panels were all concordant with the respective unspiked control.

14.11 Hook effect

No high-dose hook effect was observed up to a S/CO value of 2990.

15. REFERENCES

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The symbols glossary is provided electronically and can be found in the Dialog section at www.diasorin.com using the part and lot numbers associated with the corresponding IVD product.

LIAISON® XL murex Control HBsAg Qual ([REF] 318251)

1. INTENDED USE

The LIAISON® XL murex Control HBsAg Qual (negative and positive) is intended for use as assayed quality control samples to monitor the performance of the LIAISON® XL murex HBsAg Qual assay. The performance characteristics of LIAISON® controls have not been established for any other assays or instrument platforms different from LIAISON® XL.
For details, refer to the Analyzer Operator's Manual.

Caution: **U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner**.

2. MATERIALS PROVIDED

Negative control (2 x 4,0 mL)	**[CONTROL|-]**	Human plasma non-reactive for hepatitis B surface antigen and antibody, 0.2% ProClin® 300, preservatives.
Positive control (2 x 4,0 mL)	**[CONTROL|+]**	Human serum reactive for hepatitis B surface antigen, 0.2% ProClin® 300, preservatives.

ProClin® is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

All reagents are supplied ready to use. The range of values of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs. Each laboratory is responsible for adopting different limits to meet individual requirements.

The certificate of analysis bar codes give specific information on the lot of controls and should be read by the hand-held bar code scanner of the LIAISON® XL Analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

3. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- Controls are not kit lot specific and may be safely interchanged even with different reagent integral lots.
- All materials used to produce the components provided in this kit have been tested for the presences 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.
- 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.
- Observe the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- 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.

Chemical Hazard and Safety Information
Reagents in this kit are classified in accordance with the US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and European Union EC Regulation 1272/2008 (CLP) (for additional information see Safety Data Sheet available on www.diasorin.com).

Hazardous reagents are classified and labelled as follows:

REAGENTS:	[CONTROL|-], [CONTROL|+]
CLASSIFICATION:	Skin sens. 1 H317
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction.
PRECAUTIONARY STATEMENTS:	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.
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

4. STORAGE AND STABILITY

Upon receipt, the controls must be stored in an upright position to prevent adherence of the solution to the vial cap. Do not freeze. When controls are stored, sealed and kept upright, they are stable at 2-8°C up to the expiry date. After removing the seals, the control vial is stable for twelve (12) weeks when stored upright at 2-8°C. Avoid bacterial contamination of controls. The controls should not be used past the expiry date indicated on the vial labels.

5. PREPARATION OF REAGENTS

– Place the control vials in type C racks on the analyzer. Each control solution allows at least 20 tests to be performed.
– The minimum volume required is 550 µL (150 µL control + 400 µL dead volume).
– At the time of use, equilibrate controls to room temperature (20-25°C) before opening the vials and keep them on board the instrument only for the amount of time required for quality control testing.
– After use, stopper the vials promptly and store them at 2-8°C in an upright position.
– During handling, use appropriate precautions to avoid bacterial contamination of controls.

6. TARGET VALUES

The range of concentration of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs. If control values obtained after successful calibration lie repeatedly outside the expected ranges, the test should be repeated using an unopened control vial.

7. QUALITY CONTROL

Quality control should be performed once per day of use, or according to guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI document, C24-A3, and 42 CFR 493.1256(c) for guidance on appropriate quality control practices. LIAISON® controls are intended to monitor for reagent failure. Whenever LIAISON® controls lie outside the expected ranges, calibration should be repeated and controls and samples retested. Do not report patient results until control results are within expected ranges. Strict adherence to the instructions of the LIAISON® XL MUREX HBsAg Qual kit are necessary to obtain reliable results.

8. LIMITATIONS

The LIAISON® XL MUREX Control HBsAg Qual positive control will not ensure precision at the assay cut-off.
Control values for assays other than the LIAISON® XL MUREX HBsAg Qual assay have not been established.
If users wish to use this control material with other assays, it is their responsibility to establish appropriate ranges.

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The symbols glossary is provided electronically and can be found in the Dialog section at www.diasorin.com using the part and lot numbers associated with the corresponding IVD product.

LIAISON® XL murex HBsAg Confirmatory ([REF] 318110)

1. INTENDED USE

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 Qual ([REF] 318250).

Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner.

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 in order 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

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, 0.1 g/L gentamicin sulphate, 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, 0.1 g/L gentamicin sulphate
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. EQUIMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

LIAISON® XL MUREX HBsAg Qual ([REF] 318250).

LIAISON® XL MUREX HBsAg Qual negative and positive controls ([REF] 318251).
LIAISON® XL Cuvettes ([REF] X0016).
LIAISON® XL Disposable Tips ([REF] X0015).
LIAISON® XL Starter Kit ([REF] 319200).
LIAISON® Wash/System Liquid ([REF] 319100).
LIAISON® XL Waste Bags ([REF] X0025).

6. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

- Do not mix reagents or exchange components from kits with different lot numbers.
- Diagnostic performance of LIAISON® XL MUREX HBsAg Confirmatory ([REF] 318110) have been established only when used in combination with LIAISON® XL MUREX HBsAg Qual ([REF] 318250) and LIAISON® XL MUREX Controls HBsAg Qual ([REF] 318251).
- Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen in the CDCNIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 2007, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses 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, biological reagents and materials used in the assay must be considered 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. 1 H317
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction.
PRECAUTIONARY STATEMENTS:	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.
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

7. 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 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.

8. SPECIMEN COLLECTION AND PREPARATION

For specimen collection and preparation refer to Instruction for Use of LIAISON® XL MUREX HBsAg Qual ([REF] 318250).
The minimum volume of specimen required for this confirmatory assay is 700 µL for slightly reactive samples (e.g., S/CO value <50) and 20 µL for highly reactive samples (e.g. S/CO value > 50).

9. ASSAY PROCEDURE

Test the LIAISON® XL MUREX HBsAg Qual 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 positive patient samples (S/CO < 50).

Specimen treatment

1. POSITIVE CONTROL AND SLIGHTLY REACTIVE SAMPLES (value < 50 S/CO):
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.

2. HIGHLY REACTIVE SAMPLES (value > 50 S/CO):
Dilute specimens 1:10,000 with LIAISON® XL MUREX HBsAg Confirmatory specimen diluent before testing. Operate as follows:
Dispense 4 µL of each specimen and 36 µL specimen diluent into test tubes and thoroughly mix with a Vortex to ensure adequate mixing (1:10 intermediate predilution).
Dispense 4 µL of the 1:10 intermediate predilution and 36 µL specimen diluent into clean test tubes and thoroughly mix with a Vortex to ensure adequate mixing (1:100 intermediate predilution).
Dispense 7 µL of the 1:100 intermediate predilution and 693 µL specimen diluent into clean test tubes and thoroughly mix with a Vortex to ensure adequate mixing (1:10,000 predilution). The predilutions may be stored at 2-8°C for 24 hours.
Mix 330 µL diluted specimen and 33 µL anti-HBs antibodies in one tube (neutralized aliquot) as well as 330 µL diluted specimen and 33 µL specimen diluent in another tube (non-neutralized aliquot).
Incubate the specimens for minimum one hour at room temperature (20-25°C).
If the index value of the 1:10,000 predilution is still greater than 150 S/CO for the non-neutralized aliquot, repeat the test after further diluting the specimen 1:20 (e.g., 35 µL of the 1:10,000 predilution + 665 µL specimen diluent).

If the index value of the 1:10,000 predilution is less than 0.9 S/CO for the non-neutralized aliquot, repeat the test on the 1:100 intermediate predilution of the specimen.
Operate as follows: Dispense 7 µL of each specimen and 693 µL specimen diluents into test tubes and thoroughly mix with a Vortex to ensure adequate mixing.

Assay procedure For Use with the LIAISON® XL MUREX HBsAg Qual ([REF] 318250) on the LIAISON® XL Analzyer

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 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 Qual 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 Qual control set into the reaction cuvettes.
3. Dispense specimens in duplicate (i.e., neutralized and non-neutralized aliquots) into the reaction cuvettes.
4. Dispense coated magnetic particles into the reaction cuvettes.
5. Dispense Buffer L into the reaction cuvettes.
6. Incubate.
7. Dispense conjugate into the reaction cuvettes.
8. incubate
9. Wash with Wash/System liquid.
10. Add the Starter Reagents and measure the light emitted.

10. QUALITY CONTROL

Quality control is suggested once per day of use, or according to guidelines or requirements of local regulations or accredited organizations. Non-treated LIAISON® XL MUREX Control HBsAg Qual ([REF] 318251) (Negative and Positive) must be tested in every LIAISON® XL MUREX HBsAg Confirmatory run.
The Positive control must also be tested in parallel, following the same Specimen treatment procedure used for repeatedly reactive positive patient samples (S/CO < 50) as a performance check. See Assay Procedure Section 10.
The LIAISON® controls are intended to monitor for reagent failure. 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 quality control materials used.

11. LIMITATIONS OF THE PROCEDURE

- Samples having a very high HBsAg concentration can give a lower S/CO in the LIAISON® XL MUREX HBsAg Qual assay. These samples are not adequately neutralized by the confirmatory reagent, and are not confirmed as positive. These samples are recognisable as the S/CO value of the sample treated with the confirmatory reagent is higher than the S/CO value for the non-neutralized sample as a result of the dilution effect. The confirmation testing of these samples should be repeated, by pre-diluting the samples (1:200 000).
- 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.

12. 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.

13. INTERPRETATION OF RESULTS

The test is valid if neutralization of the LIAISON® XL MUREX HBsAg Qual positive control ([REF] 318251) 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%.

A specimen should be further diluted with LIAISON® XL MUREX HBsAg Qual specimen diluent (up to 1:200,000) and neutralization repeated when the value of the non-neutralized aliquot (mixed with specimen diluent) is greater than 50 S/CO (neat specimen) or 150 (diluted specimen) and percent neutralization is less than 50%.

Specimen dilution	S/CO (non-neutralized aliquot)	% Neutralization	Interpretation of results
Neat	< 0.9 0.9 - 50 ≥ 50	Any value < 50% ≥ 50%	Not confirmed (HBsAg-negative specimen). Not confirmed (interfering substance). Confirmed (true HBsAg-positive specimen).
1:10,000	< 0.9 0.9 -150 ≥ 0.9 ≥ 150	Any value < 50% ≥ 50% < 50%	Retest 1:100 dilution. Not confirmed (interfering substance). Confirmed (true HBsAg-positive specimen). Further dilute (1:200,000) and retest.
1:100	0.9 -150 ≥ 0.9	<50% ≥ 50%	Not confirmed (interfering substance). Confirmed (true HBsAg-positive specimen).
1:200,000	0.9 -150 ≥ 0.9	<50% ≥ 50%	Not confirmed (interfering substance). Confirmed (true HBsAg-positive specimen).

14. SPECIFIC PERFORMANCE CHARACTERISTICS

Three hundred eighteen (318) samples from the 3882 samples tested in the clinical study were initially reactive for HBsAg with the LIAISON® XL MUREX HBsAg Qual assay. Samples were retested in duplicate as per the Instructions for Use. Of the 318 initially reactive samples 311 were repeatedly reactive. The 311 repeatedly reactive samples were then tested using the LIAISON® XL MUREX HBsAg Confirmatory assay following the recommended dilutions (neat, 1:100, 1:10,000 or 1:200,000).

HBV classification	**N**	**LIAISON® XL MUREX HBsAg Qual Repeatedly Reactive**	**LIAISON® XL MUREX HBsAg Confirmatory Confirmed Reactive**
Acute	109	106	106
Late Acute	34	34	33
Chronic	144	144	144
Early Recovery	57	2	0
Recovery	167	17	4
Immune Due to Natural Infection	107	0	0
HBV Vaccine Response	1152	0	0
Not Previously Infected	1303	3	2
Not Interpretable	9	0	0
Pregnancy	800	5	4
Total	3775	311	293/311 = 94.2%

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