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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 Anti-HBc ([REF] 318130)

1. INTENDED USE

The LIAISON® XL murex Anti-HBc assay is an *in vitro* chemiluminescent immunoassay (CLIA) for the qualitative detection of IgG and IgM (total) antibodies to hepatitis B core antigen (anti-HBc) in human adult and pediatric (2 – 21 years) serum and plasma (lithium and sodium heparin, sodium citrate and K_2 EDTA), including separator tubes, on the LIAISON® XL Analyzer. Assay results in conjunction with other laboratory results and clinical information may be used as an aid in the diagnosis of hepatitis B virus (HBV) infection in patients with symptoms of hepatitis or who may be at risk for HBV infection.

This assay is not intended 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

Hepatitis B is a liver disease caused by infection with the hepatitis B virus (HBV). The HBV is an enveloped deoxyribose nucleic acid (DNA) virus. Inside the envelope is the inner core of the virus which contains the hepatitis B core antigen (HBcAg). Following an infection with the 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 transmitted from person to person by contact with infected body fluids. Most infections occur by coming into 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 be transmitted also 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 an 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 mortality. HBV is endemic throughout the world, despite the availability of an efficient vaccine (4, 5, 6).

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 often 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, to assess the clinical phases of the disease, to monitor antiviral therapy and the immune status of the patient (7-11).

Anti-HBc antibodies can be detected during both the acute and chronic stages of HBV infection. In contrast, some assays will only detect the IgM isotypes that typically occur during acute viral infection and decline to undetectable levels within 6 months; in contrast, the IgG isotype of anti-HBc, in the absence of HBsAg, persists indefinitely and could be the only detectable serological marker after an acute HBV infection (12). Further, due to the lifelong duration of anti-HBc antibodies, they are considered the most sensitive and constant marker of HBV exposure regardless of acute or chronic infection status (13; 14).

The total anti-HBc serological test plays an important role in detecting occult HBV infections (15). The presence of the IgG isotype of anti-HBc along with HBsAg-negative status typically indicates a past HBV infection; this status can indicate there is a risk of HBV transmission (occult HBV infection) (15-16). Total anti-HBc assays are particularly useful to differentiate occult infections in individuals that have an undetectable anti-HBs response and intermittently detectable HBV DNA through nucleic acid testing (NAT). Anti-HBc results are often followed up with additional testing for anti-HBs, anti-HBe or HBeAg assays.

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of anti-HBc is a two-step competitive chemiluminescence immunoassay (CLIA). Recombinant HBcAg is used for coating magnetic particles (solid phase) and antibodies to HBcAg (mouse monoclonal) are linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, anti-HBc present in calibrators, samples or controls binds to a fixed and limited amount of recombinant HBcAg bound to the solid phase. During the second incubation, the antibody conjugate links the unbound solid-phase recombinant HBcAg epitopes. After each incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is inversely indicative of anti-HBc concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	**[SORB]**	Magnetic particles coated with HBcAg obtained in *E. coli* by the recombinant DNA technology, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (1.8 mL)	**[CAL|1]**	Calf serum containing high anti-HBc levels, 0.2% ProClin® 300, preservatives.
Calibrator 2 (1.8 mL)	**[CAL|2]**	Human serum without anti-HBc antibodies, 0.2% ProClin® 300, preservatives, an inert blue dye.
Buffer F (11 mL)	**[BUF|F]**	Acetate buffer, EDTA.
Conjugate (23 mL)	**[CONJ]**	Antibodies to HBcAg (mouse monoclonal) conjugated to an isoluminol derivative, human serum, newborn calf serum, phosphate buffer, EDTA, 0.2% ProClin® 300, preservatives, an inert blue dye.
Number of tests		100

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 Control Anti-HBc ([REF] 318131).

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For Prescription Use Only.
- The human blood source material used to produce the components provided in this kit derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 when tested by an FDA-approved method and found to be non-reactive for syphilis when tested by a serological test. 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 are 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.

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:

Hazardous reagents are classified and labelled as follow:**REAGENTS:**	[CAL|1], [CAL|2], [CONJ]
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).

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 Preventions, Atlanta, GA, 1976.

Pursuant to EC Regulation 1272/2008 (CLP), [SORB] and [BUF|F] are 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 suspension:

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

Repeat as necessary until the magnetic particles are completely resuspended.

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

Loading of integral into the reagent area

– LIAISON® 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 Reagent Integral Preparation 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 and Sodium Heparin, whereas the Sodium Citrate plasma can lead to lower Index values (about 10%) in comparison to normal serum.

Blood should be collected aseptically by venipuncture, allowed to clot (if applicable), and the serum or plasma 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 four (4) days does not influence the assay performance. If the assay is performed within four (4) 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) for up to three (3) months. If samples are stored frozen, mix thawed samples well before testing. Samples are stable through three (3) 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 260 µL (110 µL specimen + 150 µL dead volume). Dead volume is the volume left at the bottom of the aliquot tube which the instrument cannot aspirate.

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 four (4) 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 module.
2. Dispense buffer F.
3. Dispense coated magnetic particles.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid
9. Add the Starter Kit and measure the light emitted.

10. CALIBRATION

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows four (4) calibrations to be performed.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

– A new lot of reagent integral or of 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 provided on the control vial labels, 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 patient 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.

Test results are reported qualitatively as Reactive or Non-reactive for the presence of anti-HBc. However, diagnosis of infectious diseases should not be established on the basis of a single test result but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgement.

Specimens collected in sodium citrate may yield lower Index values (about 10%) in comparison to normal serum which may cause an increased likelihood of false negative results.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates anti-HBc levels expressed as index value and grades the results.

For details, refer to the analyzer operator's manual.

The interpretation of results for the LIAISON® XL MUREX Anti-HBc is as follow:

- the cut-off index value of 1.00 determines whether a sample has detectable levels of anti-HBc.
- **Non-Reactive**: Samples with anti-HBc levels equal to or above an index value of 1.10 are considered Non-reactive and presumed negative for Anti-HBc.
- **Reactive**: Samples with anti-HBc levels below an index value of 0.900 are considered Reactive and presumed positive for Anti-HBc.
- Samples with anti-HBc levels ranging between an index value of 0.900 and 1.10 should be graded initially equivocal. Initially equivocal samples must be retested in duplicate before reporting results. Samples that are repeatedly equal to or above 1.00 (i.e. at least 2 out of 3 results) are considered Non-reactive and presumed negative for Anti-HBc. Samples that are repeatedly below 1.00 (i.e. at least 2 out of 3 results) are considered Reactive and presumed positive for Anti-HBc.

The result should be assessed in conjunction with the patient's medical history, clinical examination and other laboratory findings to determine disease status.

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1 Summary of clinical performance

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

Demographics of Clinical Study Samples by Gender

	Adult				Pediatric (0-21)				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	6.9%	1	50.0%	0	0.0%
Male	1017	38.2%	151	73.3%	61	37.9%	27	93.1%	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%**	**29**	**100.0%**	**2**	**100.0%**	**21**	**100.0%**

Demographics of Clinical Study Samples by Race

	Adult				Pediatric (0-21)				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%	25	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%**	**29**	**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 test results of the FDA approved hepatitis-B assays listed below 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		
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		

HBV Classification	HBsAg	HBeAg	Anti-HBc	Anti-HBc IgM	Anti-HBe	Anti-HBs	Prospective (n)	Retrospective (n)
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 Anti-HBc results for the 2826 prospective and 256 retrospective specimens were compared to a reference Anti-HBc assay. The following tables show this comparison and percent agreement with 95% exact confidence intervals with the reference anti-HBc assay results.

Cumulative Clinical Agreement (Combined Prospective & Retrospective*)
LIAISON® XL MUREX Anti-HBc vs Reference Assay by Characterization

HBV Classification	Reference Anti-HBc assay				Total
	Reactive		Non-reactive		
	LIAISON® XL MUREX Anti-HBc		LIAISON® XL MUREX Anti-HBc		
	Reactive	Non-reactive	Reactive	Non-reactive	
Acute	100	0	2	7	109
Late Acute	34	0	0	0	34
Chronic	142	2	0	0	144
Early Recovery	52	5	0	0	57
Recovery	162	1	1	3	167
Immune Due to Natural Infection	99	8	0	0	107
HBV Vaccine Response	1	0	30	1121	1152
Not Previously Infected	0	0	6	1297	1303
Not Interpretable	2	0	0	7	9
Total	**592**	**16**	**39**	**2435**	**3082**

*An analysis of study data showed no significant difference in assay performance between prospective and retrospective specimens

Cumulative Clinical Agreement (Combined Prospective & Retrospective)
LIAISON® XL MUREX Anti-HBc Total vs Reference Assay by Characterization

HBV Classification	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
Acute	100/100 (100.0%)	7/9 (77.8%)

HBV Classification	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
	95% CI: 96.3% to 100.0%	95% CI: 45.3% to 93.7%
Late Acute	34/34 (100.0%) 95% CI: 89.8% to 100.0%	N/A
Chronic	142/144 (98.6%) 95% CI: 95.1% to 99.6%	N/A
Early Recovery	52/57 (91.2%) 95% CI: 81.1% to 96.2%	N/A
Recovery	162/163 (99.4%) 95% CI: 96.6% to 99.9%	3/4 (75.0%) 95% CI: 30.1% to 95.4%
Immune Due to Natural Infection	99/107 (92.5%) 95% CI: 85.9% to 96.2%	N/A
HBV Vaccine Response	1/1 (100.0%) 95% CI: 20.7% to 100.0%	1121/1151 (97.4%) 95% CI: 96.3% to 98.2%
Not Previously Infected	N/A	1297/1303 (99.5%) 95% CI: 99.0% to 99.8%
Not Interpretable	2/2 (100.0%) 95% CI: 34.2% to 100.0%	7/7 (100.0%) 95% CI: 64.6% to 100.0%

Cumulative Pediatric Clinical Agreement (Combined Prospective & Retrospective)
LIAISON® XL MUREX Anti-HBc Total 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	1/1 (100.0%) 95% CI: 20.7% to 100.0%	N/A
Recovery	5/5 (100.0%) 95% CI: 56.6% to 100.0%	N/A
Immune Due to Natural Infection	3/3 (100.0%) 95% CI: 43.9% to 100.0%	N/A
HBV Vaccine Response	N/A	62/63 (98.4%) 95% CI: 91.5% to 99.7%
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%

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) months to twenty-one (21) years. Ten (10) negative pediatric samples were spiked with IgG anti-HBc high positive sample to obtain high negative samples. Ten (10) pediatric samples were spiked with IgG anti-HBc high positive sample to obtain low positive samples. Ten (10) pediatric samples were spiked with IgG anti-HBc high positive sample to obtain moderate positive samples. Adult negative pool samples were used as controls, by spiking with IgG anti-HBc 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. Within Laboratory 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 Anti HBc 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.

Sample ID	N	Mean	Repeatability (within-run)		Between Run		Between Day		Between Lot		Within Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Neg #RS-672	240	*156933	2455	1.6%	4303	2.7%	3288	2.1%	4868	3.1%	7684	4.9%
Ctrl Neg #RS-673	240	*155995	2351	1.5%	4801	3.1%	886	0.6%	4729	3.0%	7192	4.6%
Ctrl Neg #RS-674	240	*155885	2161	1.4%	4524	2.9%	2073	1.3%	4378	2.8%	6972	4.5%
Ctrl Pos #RS-675	240	0.368	0.007	1.9%	0.014	3.8%	0.012	3.2%	0.029	7.8%	0.035	9.5%
Ctrl Pos #RS-676	240	0.369	0.006	1.6%	0.016	4.3%	0.011	3.0%	0.029	7.8%	0.035	9.5%
Ctrl Pos #RS-677	240	0.389	0.007	1.8%	0.018	4.7%	0.015	3.8%	0.036	9.3%	0.044	11.2%
AHBC-1-U1	240	2.31	0.032	1.4%	0.062	2.7%	0.053	2.3%	0.04	1.7%	0.097	4.2%
AHBC-1-U2	240	1.50	0.031	2.1%	0.075	5.0%	0.042	2.8%	0.08	5.3%	0.122	8.1%
AHBC-1-U3	240	1.58	0.036	2.3%	0.076	4.8%	0.047	3.0%	0.079	5.0%	0.125	7.9%
AHBC-1-U4	240	1.46	0.033	2.2%	0.073	5.0%	0.047	3.2%	0.084	5.8%	0.125	8.6%
AHBC-1-U5	240	0.754	0.016	2.1%	0.033	4.3%	0.024	3.2%	0.054	7.2%	0.07	9.2%
AHBC-1-U6	240	0.773	0.017	2.2%	0.037	4.8%	0.029	3.8%	0.063	8.2%	0.081	10.4%
AHBC-1-U7	240	0.586	0.013	2.2%	0.031	5.3%	0.018	3.1%	0.044	7.5%	0.058	10.0%
AHBC-1-U8	240	0.174	0.003	1.9%	0.008	4.6%	0.005	2.9%	0.02	11.5%	0.022	12.9%
AHBC-1-U9	240	0.237	0.004	1.5%	0.007	2.9%	0.005	2.3%	0.015	6.3%	0.018	7.4%
AHBC-1-U10	240	0.262	0.005	1.8%	0.007	2.8%	0.005	1.8%	0.017	6.5%	0.02	7.5%

* Samples below the reading range of the assay, precision calculations are based on signal (RLU)

14.4 Reproducibility

A 5-day reproducibility/precision study was conducted at two (2) external laboratories and one (1) internal DiaSorin laboratory. Each site used a different lot of LIAISON® XL MUREX Anti-HBc 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	Mean	Repeatability (within-run)		Between Days/Runs		Within Laboratory		Between Sites/Lots		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Neg (all 3 lots)	90	*131264	3373	2.6%	4696	3.6%	5782	4.4%	3421	2.6%	6718	5.1%
Ctrl Pos (all 3 lots)	90	0.378	0.008	2.1%	0.016	4.3%	0.018	4.8%	0.013	3.4%	0.022	5.9%
AHBC-1-U1	90	2.336	0.045	1.9%	0.092	3.9%	0.103	4.4%	0.092	4.0%	0.138	5.9%
AHBC-1-U2	90	1.547	0.063	4.0%	0.133	8.6%	0.147	9.5%	0.144	9.3%	0.206	13.3%
AHBC-1-U3	90	1.624	0.061	3.8%	0.092	5.7%	0.111	6.8%	0.097	5.9%	0.147	9.0%
AHBC-1-U4	90	1.464	0.060	4.1%	0.084	5.8%	0.104	7.1%	0.126	8.6%	0.163	11.2%
AHBC-1-U5	90	0.769	0.031	4.0%	0.049	6.4%	0.058	7.6%	0.044	5.7%	0.073	9.5%
AHBC-1-U6	90	0.807	0.036	4.4%	0.063	7.8%	0.072	8.9%	0.025	3.1%	0.076	9.5%
AHBC-1-U7	84	0.595	0.023	3.8%	0.04	6.7%	0.046	7.7%	0.022	3.7%	0.051	8.6%
AHBC-1-U8	90	0.184	0.007	3.8%	0.018	9.8%	0.019	10.5%	0.016	8.9%	0.025	13.8%

AHBC-1-U9	90	0.241	0.005	2.1%	0.009	3.6%	0.010	4.2%	0.016	6.7%	0.019	7.9%
AHBC-1-U10	90	0.260	0.007	2.7%	0.004	1.6%	0.008	3.1%	0.011	4.1%	0.013	5.2%

*Precision calculations are based on signal (RLU) for the negative control.

14.5. Analytical Sensitivity as Seroconversion Panel Performance

Nine (9) commercially available HBV seroconversion panels were tested using LIAISON® XL MUREX Anti-HBc and a commercially available FDA-approved comparator assay to determine the sensitivity of the assay. The results are summarized in the following table:

PANEL ID	Number of Samples	LIAISON® XL MUREX Anti-HBc		ETI-AB-COREK PLUS (Reference test)		Difference in days	Difference in number of blood draws
		Last Bleed (Draw) with NR* Result	First Bleed (Draw) with R** Result	Last Bleed (Draw) with NR* Result	First Bleed (Draw) with R**Result		
HBV-001	20	13	29	29	31	-2	-1
HBV-004	30	65	71	76	78	-7	-2
HBV-002	20	25	57	57	60	-3	-1
9092	37	78	85	92	99	-14	-2
9072	17	154	159	152	154	5	1
6278	10	33	41	33	41	0	0
6281	12	36	41	36	41	0	0
9093	31	42	49	42	49	0	0
9099	20	51	61	78	82	-21	-3
TOT	**197**	**497**	**593**	**595**	**635**	**-42**	**-8**

The sensitivity of the LIAISON® XL MUREX Anti-HBc was comparable to the comparator assay in the nine seroconversion panels tested. The LIAISON XL MUREX Anti-HBc assay yielded a reactive result sooner by one blood draw or more than the comparator assay in 5 panels, a reactive result in the same blood draw as the comparator assay in 3 panels and yielded a reactive result one blood draw later than the comparator method in one panel.

14.6. 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 Anti-HBc assay. Each sample was divided into three aliquots. Two sets of aliquots were spiked with an IgG anti-HBc 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 equivalent assay performance among serum (with and without gel SST), K_2 EDTA, lithium heparin and sodium heparin, whereas samples collected in sodium citrate plasma can lead to approximately 10% lower Index values in comparison to normal serum.

14.7 Potential interfering substances

Controlled studies of potentially interfering substances at five (5) Anti HBc levels showed no interference at the concentration for each substance listed below in the LIAISON® XL MUREX Anti-HBc assay. The testing was based on CLSI-EP07.

Substances	Tested concentrations
Triglycerides	3000 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	20 mg/dL
Conjugated bilirubin	20 mg/dL
Albumin	6000 mg/dL
Cholesterol	350 mg/dL

14.8 Cross-Reactivity

The LIAISON® XL MUREX Anti-HBc assay was evaluated for potential interference with specimens from individuals with medical conditions unrelated to hepatitis B infection, and from other conditions that may result from atypical immune system

activity (i.e. rheumatoid factor, anti-nuclear antibodies, HAMA). All the specimens tested negative with a comparator anti-HBc assay. None of the specimens tested reactive with the LIAISON® XL MUREX Anti-HBc assay. There is no evidence of cross reactivity with the tested medical conditions.

Organism / Condition	N	Comparator Anti HBc assay	LIAISON® XL MUREX Anti-HBc	
			Non reactive	Reactive
Anti-nuclear antibodies (ANA)	10	Negative	10	0
Auto-immune hepatitis	10	Negative	10	0
C. trachomatis	11	Negative	11	0
CMV (IgG / IgM)	11	Negative	11	0
EBV (IgM)	11	Negative	11	0
Fatty liver disease	11	Negative	11	0
HAMA	11	Negative	11	0
Hemodialysis patient	11	Negative	11	0
Hepatitis A Virus (anti-HAV IgM)	11	Negative	11	0
Hepatitis C Virus (anti-HCV)	11	Negative	11	0
Hepatocellular carcinoma	11	Negative	11	0
HIV-1 (anti-HIV-1)	11	Negative	11	0
HIV-2 (anti-HIV-2)	11	Negative	11	0
HSV (IgG / IgM)	11	Negative	11	0
HTLV-1/2 (anti-HTLV)	11	Negative	11	0
IgG monoclonal gammopathy	11	Negative	11	0
IgM monoclonal gammopathy	10	Negative	10	0
Influenza vaccine recipients	11	Negative	11	0
Multiparous pregnancies	11	Negative	11	0
Multiple myeloma	11	Negative	11	0
Multiple transfusion recipients	11	Negative	11	0
N. gonorrhoea	11	Negative	11	0
Pregnancy 1st trimester	11	Negative	11	0
Pregnancy 2nd trimester	10	Negative	10	0
Pregnancy 3rd trimester	11	Negative	11	0
Rheumatoid Factor	11	Negative	11	0
T. pallidum	11	Negative	11	0
T.cruzi (anti-T. cruzi)	11	Negative	11	0

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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 Anti-HBc ([REF] 318131)

1. INTENDED USE

The LIAISON® XL MUREX Control Anti-HBc (negative and positive) is intended for use as assayed quality control samples to monitor the performance of the LIAISON® XL MUREX Anti-HBc assay. The performance characteristics of LIAISON® XL MUREX Control Anti-HBc have not been established for any other assays or instrument platforms.

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 2.7 mL)	**[CONTROL|-]**	Human serum without HBc antibodies with 0.2% ProClin® 300 and preservatives.
Positive control (2 x 2.7 mL)	**[CONTROL|+]**	Human serum containing HBc antibodies (human), 0.2% ProClin® 300 and 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 concentrations 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 on the control vial 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 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.
- 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 follow:

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 at 2-8°C 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. Once opened, controls are stable for twelve weeks (12) when properly stored at 2-8°C between multiple uses. 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 510 µL (110 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® XL controls are intended to monitor for reagent failure. Whenever LIAISON® XL controls lie outside the expected ranges provided on the certificate of analysis, 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 Anti-HBc kit are necessary to obtain reliable results.

8. LIMITATIONS

Control values for assays other than the LIAISON® XL MUREX Control Anti-HBc assay have not been established.

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