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Changes: Update of Legal Manufacturer Name;
Deletions: -

LIAISON® XL murex HCV Ab (REF 310240)

1. INTENDED USE

The LIAISON® XL MUREX HCV Ab assay uses chemiluminescence immunoassay (CLIA) technology for the qualitative determination of specific antibodies to hepatitis C virus (anti-HCV) in human serum or plasma samples.
The test has to be performed on the LIAISON® XL analyzers only.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV) was identified in 1988 as the main aetiological agent of non-A, non-B (NANB) hepatitis, accounting for 80-90% of post-transfusion hepatitis cases. HCV is a single-stranded positive-sense RNA virus, globally distributed.
Patients infected with HCV may initially present a mild or even asymptomatic acute stage of the disease; however, over 80% of the individuals who contract the disease develop chronic hepatitis and in the long run can acquire liver cirrhosis and run an increased risk of hepatocellular carcinoma.
HCV is transmitted primarily through parenteral routes, like blood transfusion, haemodialysis and intravenous drug use. HCV antibodies have not only been found in patients with acute or chronic forms of hepatitis C, but also in many asymptomatic donors after seroconversion of the recipient.

The screening of HCV antibodies is aimed at curbing the risk of transmitting HCV infection, although the presence of HCV antibodies is not a diagnosis of hepatitis C.

This immunoassay employs HCV polypeptides able to recognize antibodies directed to HCV. The polypeptides correspond to highly antigenic determinants of both the structural and non-structural regions of HCV.

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of specific IgG to hepatitis C virus (HCV) is an indirect chemiluminescence immunoassay (CLIA). Two recombinant antigens (core and NS4) specific for HCV are used for coating magnetic particles (solid phase), while a third HCV antigen (biotinylated NS3) is provided lyophilized, as a separate reagent. During the first incubation, the biotinylated antigen is captured by streptavidin-coated magnetic particles, and HCV antibodies present in calibrator, samples or controls bind to the solid phase through the recombinant HCV antigens. During the second incubation, a mouse monoclonal antibody to human IgG, linked to an isoluminol derivative (isoluminol-antibody conjugate), reacts with IgG to HCV already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.
Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IgG to HCV presence in calibrator, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles coated with HCV core and NS4 recombinant antigens (obtained in baculovirus and *E. coli* respectively), streptavidin-coated magnetic particles, BSA, PBS buffer, EDTA, preservatives.
Calibrator (3.9 mL)	CAL	Diluted antiserum containing low anti-HCV levels, BSA, PBS buffer, EDTA, 0.2% ProClin® 300, an inert yellow dye.
Specimen diluent (18.5 mL)	DIL SPE	BSA, casein, non-specific recombinant protein (obtained in *E. coli*), phosphate buffer, EDTA, preservatives, an inert blue dye.
Conjugate (18.5 mL)	CONJ	Mouse monoclonal IgG to human IgG conjugated to an isoluminol derivative, foetal calf serum, phosphate buffer, 0.2% ProClin® 300, preservatives, an inert red dye.
Number of tests		100

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

Included in the kit:

HCV NS3 antigen	Ag	Biotinylated HCV NS3 recombinant antigen (obtained in *E. coli*), MES buffer (lyophilized reagent, blue cap).
Buffer K (3.7 mL)	BUF K	MES buffer, preservatives (ready-to-use reagent, brown cap).

Materials required but not provided

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 HCV Ab controls (negative and positive) (REF 310241).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

All human serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive, except for the positive control, which is reactive for HCV antibodies. The units positive for HCV antibodies have been inactivated by heat treatment (60°C for one hour) during the manufacturing process. They may derive from HCV-infected patients and therefore should be considered as potentially infectious.
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.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

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

REAGENTS:	SORB	CAL, CONJ
CLASSIFICATION	Eye irrit. 2 Skin irrit. 2	Skin sens. 1 H317
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H315 Causes skin irritation. H319 Causes serious eye irritation.	H317 May cause an allergic skin reaction.
PRECAUTIONARY STATEMENTS:	P264 Wash hands thoroughly after handling. 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.	P261 Avoid breathing dust/fume/gas/mist/ vaporous/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).	n.a.	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:	Ag (lyophilized)
CLASSIFICATIONS	Skin corr. 1B H314 Eye Dam. 1 H318
SIGNAL WORD:	Danger
SYMBOLS / PICTOGRAMS:	 GHS05 Corrosion
HAZARD STATEMENTS:	H314 Causes severe skin burns and eye damage.
PRECAUTIONARY STATEMENTS:	P260 Do not breathe dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	tris(2-carboxyethyl)phosphine hydrochloride.

Note: after reconstitution, Ag is classified not hazardous.
Pursuant to EC Regulation 1272/2008 (CLP), BUF K is labeled as EUH210 safety data sheets available on request.

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

7. REAGENT PREPARATION

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:
Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Carefully wipe the surface of each septum to remove residual liquid.
Repeat as necessary until the magnetic particles are completely resuspended.

An incomplete magnetic particles resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:
Visually inspect the reagents, calibrator in particular (position two 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. Load the integral into the reagent area once the foam has dissipated.

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.

HCV NS3 ANTIGEN

NS3 antigen for LIAISON® XL MUREX HCV Ab assay is supplied lyophilized. The reagent is kit lot specific and must be used only with the reagent integral lot it is matched with. Correct lot matching between reagent integral and NS3 antigen is automatically checked by the LIAISON® XL analyzer. The reagent allows at least 100 tests to be performed. **Do not pool the contents of different NS3 antigen vials, even if they belong to the same lot**.

– Reconstitute the vial contents with 3.5 mL buffer K.
– Mix vial thoroughly by gentle inversion after sealing with stopper and cap. Avoid foaming.
– Allow the vial to stand for 10-15 minutes at 18-25°C to achieve complete dissolution.
– The reconstituted antigen solution must be loaded on to the instrument in the ancillary reagent area, immediately before use. After use, replace the cap and store at 2-8°C. Once opened and reconstituted, the reagent is stable for four weeks when properly stored at 2-8°C between two successive uses.

For details on the reagent use in the ancillary reagent area on board the instrument, refer to the LIAISON® XL operator's manual.
Vial label refers only to lyophilized Ag. Once reconstituted, pursuant to EC Regulation 1272/2008 (CLP), Ag is classified as not hazardous.

CONTROLS

Refer to the LIAISON® XL MUREX HCV Ab Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY

– **Sealed**: Stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**: Stability four weeks.
– Use storage rack provided with the LIAISON® XL analyzer for upright storage of reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate later proper resuspension of magnetic particles.
– Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma may be used (including serum collected in serum separator tubes). The anticoagulants sodium citrate, potassium EDTA, lithium and sodium heparin, potassium oxalate, ACD (acid citrate-dextrose), CPDA (citrate-phosphate-dextrose-adenine) have been tested and may be used with this assay. Post- mortem specimens, collected up to 24 hours after death, have been tested and may be also used in the assay. Preservatives with oxidative mechanism must not be added to specimens, since they may affect the immunoreactivity of recombinant proteins used to detect anti-HCV antibodies. The correct specimen type must be used in the assay.

Follow tube manufacturers' instructions carefully when using collection containers. Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation.

Centrifugation conditions range from 1,000 to 3,000 g for 10 minutes. Conditions may vary depending on tube manufacturers recommendations. Use of alternate centrifugation conditions should be evaluated and validated by the laboratory.

Before shipping specimens, serum or plasma specimens should be removed from clot, red cells or gel separator. Specimens may be shipped in dry ice (frozen), in wet ice (for 2°-8°C) or at room temperature (20°-25°C), by following sample storage limitations described below.

Uncontrolled transport conditions (in terms of temperature and time) can cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010).

Concerning storage limitations, if the assay is performed within seven days of sample collection, the samples removed from red cells, clot or gel separator may be kept at 2°-8°C; otherwise they should be aliquoted and stored deep-frozen (–20°C or below). Ten serum or plasma samples with different reactivity were stored for seven days at 2-8°C and underwent five freeze-thaw cycles. The results showed no significant differences; however multiple freeze-thaw cycles should be avoided. If samples are stored frozen, mix thawed samples well before testing.

Samples removed from red cells, clot or gel separator having particulate matter, fibrin, turbidity, lipaemia, or erythrocyte debris, specimens that have been stored at room temperature (20°-25°C), or frozen and thawed, or samples requiring repeat testing, require clarification by further centrifugation (it's recommended 10,000 g for 10') before testing, to improve consistency of results. Specimens with a lipid layer on the top should be transferred in a secondary tube, taking care to transfer only the clarified material. Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying.

The minimum volume required for a single determination is 175 µL specimen (25 µL specimen + 150 µL dead volume).

10. CALIBRATION

Assay of calibrator contained in the reagent integral allows the analyzer to set the assay cut-off. The calibrator solution allows six calibrations to be performed.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
– A new lot of Starter Kit is used.
– Whenever a new integral is used.
– The analyzer has been serviced.
– Control values lie outside the expected ranges.

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

The analyzer operations are as follows:
1. Dispense specimen diluent into the reaction cuvettes.
2. Dispense coated magnetic particles.
3. Dispense calibrator, controls or specimens.
4. Dispense reconstituted NS3 antigen.
5. Incubate.
6. Wash with Wash/System liquid.
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.

12. QUALITY CONTROL

LIAISON® XL controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® XL MUREX HCV Ab controls
(a) at least once per day of use, before running the test,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
(e) whenever a new NS3 antigen vial is used, or in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The presence or absence of HCV antibodies in the specimens is determined by comparing the chemiluminescence reaction signal to the cut-off value provided by the assay calibration. The analyzer automatically calculates the signal-to-cutoff (S/CO) ratios, then grades the results. For details, refer to the analyzer operator's manual.

Sample results should be interpreted as follows:
Specimens with signal-to-cutoff (S/CO) ratios below 1.00 are considered *non-reactive* for HCV antibodies.
Specimens with signal-to-cutoff (S/CO) ratios above or equal to 1.00 are considered *reactive* for HCV antibodies.

Specimens that show an initially reactive result should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HCV antibodies. However, like all immunoassays, the LIAISON® XL MUREX HCV Ab assay may occasionally yield non-specific reactions due to other causes. A repeatedly reactive specimen should be investigated further with sensitive, supplemental HCV-specific tests, such as immunoblot and HCV nucleic acid tests.

14. LIMITATIONS OF THE PROCEDURE

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Bacterial contamination or heat inactivation of the specimens may affect the test results.

Warning - **This test is suitable only for investigating single samples, not for diluted specimens, sample pools or heat-inactivated specimens**.

A non-reactive test result for HCV antibodies does not exclude the possibility of exposure to or infection with HCV. In fact, the subject’s antibody levels may be below the assay detection limit. 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. A full differential diagnostic work-up for the diagnosis of hepatitis C and related clinical conditions includes examination of the patient's immune status and clinical history.

Specimens from patients receiving therapeutic doses of Biotin (Vitamin H, B7 or B8) may interfere in immunoassays based on biotinylated reagents. Interference was observed testing Biotin serum concentration higher than 100ng/mL with LIAISON® XL MUREX HCV Ab assay with risk of false negative results. Such results should therefore be evaluated with care.

Before testing cadaveric specimens, collection and centrifugation procedures should be carefully applied. After death, haemolysis and other changes (including proteolysis and dilution) occur in blood, which may lead to False Negative and False Positive in testing. In subjects transfused immediately prior to death high percentage of haemodilution can affect the performance of the test due to analyte dilution.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, potassium EDTA, lithium and sodium heparin, potassium oxalate, ACD, CPDA), or by a limited number of freeze-thaw cycles of samples. Results are not influenced by the use of positive same-day fresh samples as a comparative study in 25 freshly collected specimens demonstrates.
The assay performance was not affected by the following compounds up to the tested concentrations.

Tested Compound	Tested concentration
Unconjugated bilirubin	20 mg/dL
Conjugated bilirubin	20 mg/dL
Haemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Biotin	100 ng/mL

Biotin Interference.

Doses value (S/CO) for reference condition (sample with no added biotin)	%Bias for samples containing various concentrations of Biotin				
	Biotin concentrations (ng/mL)				
	50ng/mL	100ng/mL	500ng/mL	1000ng/mL	2000ng/mL
0.34	11.8	5.9	-50.0	-85.0	-85.0
1.30	-7.7	0.0	-46.9 (FN)†	-92.8 (FN)†	-93.4 (FN)†
2.70	-11.1	-3.7	-44.5	-91.5 (FN)†	-92.6 (FN)†

†FN = False non-reactive

Specimens with biotin concentrations up to 100 ng/mL demonstrated < 10% negative bias in LIAISON® XL MUREX HCV Ab S/CO values. Biotin concentrations greater than 100 ng/mL led to higher negative bias which can cause false non-reactive LIAISON® XL MUREX HCV Ab results in samples with anti-HCV levels near the medical decision point.

The recommended daily intake for biotin is 30 µg and normal serum concentrations of biotin range from below 0.1 to 0.8 ng/mL (Grimsey, Paul, et al.: 2017 International journal of Pharmacokinetics 2.4: 247-256). High doses of biotin (up to 30 mg per day) may be taken as a dietary supplement promoted for hair, nail, or skin benefits. Some pharmacokinetic studies have shown that in subjects taking daily doses of 5 mg, 10 mg and 20 mg of biotin, serum concentrations of biotin can reach up to 73 ng/mL, 141 ng/mL and 355 ng/mL (Grimsey, Paul, et al.: 2017 International journal of Pharmacokinetics 2.4: 247-256) respectively, and it's necessary to delay sample collection after the last dose of biotin up to 73h to avoid the risk of false assay results. These studies were performed in a small number of apparently healthy subjects. Clearance of biotin could be different for other populations, for example patients with impaired renal function may have higher concentrations of biotin in serum.

Cross-reactions. The cross-reactivity study for the LIAISON® XL MUREX HCV Ab assay was designed to evaluate potential interference from antibodies to other organisms that may cause infectious diseases (EBV, hCMV, rubella virus, parvovirus B19, *Toxoplasma gondii*, *Treponema pallidum*, *Borrelia burgdorferi*, HSV, VZV, HAV, HBV, HIV, HTLV-I/II) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, human anti-mouse antibodies). Samples for these studies were pre-screened with another commercially available anti-HCV assay. If found negative for HCV antibodies, those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays. Specificity observed in potentially cross-reactive specimens is comparable to that of open populations.

Condition	Number of expected negative samples	LIAISON® XL positive results
hCMV IgG antibodies	15	0
EBV (VCA) IgG antibodies	15	0
HSV-1/2 IgG antibodies	15	0
Rubella virus IgG antibodies	15	0
Parvovirus B19 IgG antibodies	15	0
VZV IgG antibodies	15	0
HBsAg	6	0
HIV antibodies and p24 antigen	5	0
HAV antibodies	5	0
HTLV-I/II antibodies	8	0
Borrelia burgdorferi IgG antibodies	10	0
Toxoplasma gondii IgG antibodies	15	0
Treponema pallidum antibodies	13	0
Rheumatoid factor (anti-Fc immunoglobulin)	10	0
Anti-nuclear autoantibodies (ANA)	33	1
Human anti-mouse antibodies (HAMA)	16	0
E. coli antibodies	5	0
Total	216	1

15.2. Precision

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

Repeatability. Twenty replicates were performed in the same run to evaluate in-house repeatability.

Repeatability	A	B	D	E	C	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.40	1.69	1.99	2.62	2.65	0.04	3.63
Standard deviation (S/CO)	0.05	0.08	0.14	0.04	0.16	0.002	0.19
Coefficient of variation (%)	3.7	4.7	6.9	1.7	6.2	4.2	5.4
Min. value (S/CO)	1.33	1.52	1.85	2.53	2.41	0.04	3.02
Max. value (S/CO)	1.54	1.84	2.43	2.71	2.96	0.05	3.89

Reproducibility. Twenty determinations were performed in different days (one or two runs per day) with three different lots of integral to evaluate reproducibility. The tests were performed using two instruments.

Reproducibility - Instrument 1	A	B	D	E	C	Negative control	Positive control
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.21	1.53	1.74	2.32	2.54	0.03	3.16
Standard deviation (S/CO)	0.05	0.08	0.10	0.24	0.14	0.003	0.15
Coefficient of variation (%)	4.0	5.4	5.5	10.1	5.4	9.9	4.7
Min. value (S/CO)	1.13	1.41	1.60	1.81	2.33	0.02	2.85
Max. value (S/CO)	1.31	1.69	1.91	2.60	2.85	0.04	3.53
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.16	1.43	1.60	2.20	2.34	0.02	2.98
Standard deviation (S/CO)	0.08	0.12	0.12	0.18	0.18	0.003	0.20
Coefficient of variation (%)	6.5	8.6	7.7	8.2	7.7	11.2	6.8
Min. value (S/CO)	1.04	1.19	1.42	1.77	2.07	0.02	2.61
Max. value (S/CO)	1.29	1.62	1.81	2.52	2.70	0.03	3.26
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.17	1.44	1.65	2.17	2.30	0.03	2.97
Standard deviation (S/CO)	0.10	0.12	0.14	0.22	0.17	0.004	0.21
Coefficient of variation (%)	8.4	8.3	8.3	10.1	7.4	12.9	7.0
Min. value (S/CO)	1.03	1.24	1.44	1.74	1.98	0.02	2.68
Max. value (S/CO)	1.35	1.65	1.93	2.56	2.59	0.04	3.36
Inter-lot coefficient of variation (%)	6.3	7.4	7.2	9.5	6.9	11.3	6.2

Reproducibility - Instrument 2	A	B	D	E	C	Negative control	Positive control
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.11	1.37	1.49	2.03	2.19	0.02	2.82
Standard deviation (S/CO)	0.05	0.06	0.12	0.19	0.11	0.002	0.15
Coefficient of variation (%)	4.2	4.6	8.2	9.5	5.2	7.4	5.4
Min. value (S/CO)	1.03	1.17	1.09	1.48	1.96	0.02	2.60
Max. value (S/CO)	1.20	1.49	1.69	2.22	2.43	0.03	3.09
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.12	1.37	1.47	2.03	2.16	0.03	2.73
Standard deviation (S/CO)	0.08	0.09	0.11	0.16	0.11	0.01	0.16
Coefficient of variation (%)	6.8	6.4	7.1	7.7	5.0	18.4	5.8
Min. value (S/CO)	1.01	1.26	1.32	1.62	1.98	0.02	2.43
Max. value (S/CO)	1.25	1.56	1.66	2.29	2.37	0.03	3.16
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (S/CO)	1.09	1.31	1.49	2.04	2.11	0.03	2.69
Standard deviation (S/CO)	0.06	0.12	0.08	0.15	0.13	0.002	0.18
Coefficient of variation (%)	5.9	8.9	5.5	7.3	6.2	8.2	6.5
Min. value (S/CO)	1.00	1.08	1.31	1.71	1.89	0.02	2.20
Max. value (S/CO)	1.22	1.54	1.62	2.25	2.30	0.03	2.96
Inter-lot coefficient of variation (%)	5.6	6.7	7.0	8.2	5.5	11.3	5.9

15.3. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve).
The presence of possible prozone effect was evaluated by testing six high-titred samples positive for anti-HCV. All samples resulted in very high signals that would be expected with high-titred samples, indicating no sample misclassification.

15.4. Performance characteristics of cadaveric specimen testing

Performance characteristics of cadaveric specimens testing was determined by testing, according PEI validation protocol*, post-mortem specimens collected up to 24 hours after death in comparison to living donor specimens. 41 post-mortem samples were tested as unspiked and spiked at 2 levels: low positive and medium/high positive. The same procedure was performed with the same number of normal human serum from living donors, tested in parallel as reference to compare with post-mortem sample results. The results obtained were analyzed through calculation of percentage difference between mean of living donors results and mean of post-mortem results, at each reactivity level. In this study, the obtained percentage difference was equal or below 2,0% for each of the tested reactivity levels (see table below). Paired t-test analysis were performed between post-mortem and living donors specimens, spiked at low and medium/high positive levels, demonstrating not significantly difference on two groups (p value <0.05).

Repeteability was assessed using one post-mortem and one living donor specimens, spiked up to a low-level of reactivity with a human serum reactive for antibodies to hepatitis C virus (HCV). Each specimen was assessed in six replicates in the same run. The obtained percent coefficient of variation (CV%) did not exceed 15%. As reported in the table below 2.8% for the cadaveric specimen and 2.0% for the living donor were found in the study. The results refer to the group of investigated samples and are not guaranteed specifications, as differences may exist between laboratories and locations.

	Sample	Test results Means (S/CO)	Recovery (%) Post-mortem/Living donors	t-test p value	CV% 6 replicates
Neat	Post-Mortem unspiked	0.07	n.a.	n.a	n.a
	Living donors unspiked	0.08			
Low Positive	Post-Mortem spiked	1.89	-2.0	0.404	2.8
	Living donors spiked	1.93			2.0
Medium/high Positive	Post-Mortem spiked	4.23	-0.9	0.665	n.a
	Living donors spiked	4.27			

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

16. EXPECTED VALUES

Diagnostic specificity and sensitivity were estimated in accordance with the updated version of Common Technical Specification (CTS) published on Nov. 27, 2009 (Art. 5, §3 of IVD Directive 98/79/EC). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

16.1. Diagnostic specificity

A study was performed on a total of 5,274 serum and plasma specimens collected in two blood donation centres (including 100 specimens from first-time donors). Specimens tested were expected negative samples from an unselected blood donor population with zero prevalence of HCV infection. The assay shows diagnostic specificity above 99.5% (95% confidence interval: 99.51-99.83%). Additional specimens were also tested, randomly selected from hospitalized patients, dialysis patients, pregnant women, high-risk subjects (i.e., haemophiliacs, intravenous drug users, multiple transfusion recipients, and patients affected by sexually-transmitted diseases). Data of these studies are summarized in Table I (95% CI = 95% confidence interval). Positive specimens were confirmed by a reference CE-marked kit.

Table I - Diagnostic specificity.

Population	Number of cases	Initially reactive samples, No.	Repeat reactive samples, No.	Diagnostic specificity, %	Diagnostic specificity, 95% CI
Blood donors	5274	17	16	99.70 (5258/5274)	99.51-99.83
Hospitalized patients	395	4	2	99.49 (393/395)	98.18-99.94
Dialysis patients	181	3	1	99.45 (180/181)	96.96-99.99
Pregnant women	100	1	*1	100.0 (99/99)	96.34-100.0
High-risk subjects	134	2	0	100.0 (134/134)	97.29-100.0

* Specimen graded indeterminate by confirmatory test.

16.2. Diagnostic sensitivity

Diagnostic sensitivity was assessed by testing 678 specimens from preselected individuals diagnosed with acute (n = 20) or chronic HCV infection (n = 40) as well as positive HCV serology (294 of whom encompassing genotypes 1, 2, 3, 4, 4 non-a, 5, 6). Diagnostic sensitivity of this study is 100% (95% confidence interval: 99.46-100%).

In an additional study the ability of the LIAISON® XL MUREX HCV Ab assay to detect HCV antibodies was evaluated by testing sequentially-collected specimens belonging to 32 seroconversion panels from donors who seroconverted over the course of their donation history. Commercially available, precharacterized panels for HCV antibodies were used, each starting with a negative bleed and exhibiting narrow bleeding intervals. The panels were also tested by a reference CE-marked anti-HCV assay. The results show that the LIAISON® XL MUREX HCV Ab assay detected HCV antibodies two to three days (one bleed) earlier in three out of 32 panels. The reference assay detected HCV antibodies two to seven days (one bleed) earlier in three out of 32 panels. Both assays exhibited equivalent HCV antibodies detection in 26 out of 32 panels.
The test diagnostic sensitivity in the detection of HCV early infection is therefore substantially equivalent to the state-of-the-art assays.

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