DiaSorin Italia S.p.A.
Via Crescentino snc - 13040 Saluggia (VC) - Italy
www.diasorin.com
Tel. +39.0161.4871

CE 0459

Changes: Update of Legal Manufacturer name;
Deletions: -

LIAISON® XL murex HIV Ab / Ag HT (REF 310290)

1. INTENDED USE

The LIAISON® XL murex HIV Ab / Ag HT assay uses chemiluminescence immunoassay (CLIA) technology for the combined qualitative determination of p24 antigen of human immunodeficiency virus type 1 (HIV-1) and specific antibodies to both human immunodeficiency virus type 1 (group M and group O) and/or human immunodeficiency virus type 2 (HIV-2) in human serum or plasma samples.

The LIAISON® XL murex HIV Ab / Ag HT assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection and as a screening test for donated blood and plasma.
The test has to be performed on the LIAISON® XL Analyzer only.

2. SUMMARY AND EXPLANATION OF THE TEST

The aetiological agent of acquired immunodeficiency syndrome (AIDS) has been identified as two types of retrovirus, collectively designated as human immunodeficiency virus (HIV). HIV is transmitted by sexual contact between HIV-infected individuals, exposure to contaminated blood or blood products, and prenatal infection of a foetus or perinatal infection of a newborn from an infected mother. Antibodies to HIV are detected in AIDS patients and in HIV-infected asymptomatic individuals; HIV infection is always detected in AIDS patients and seropositive individuals by cell culture or after amplification of viral RNA and/or proviral DNA.

HIV-1 is classified by phylogenetic analysis into groups M (main or major), N (new, non-M, non-O), O (outlier) and P (Plantier et al., Nat. Med. 2009). The global AIDS pandemic was mainly caused by group M viruses, while group N and O viruses are relatively rare and endemic to West Central Africa. However, group O infections have been identified in Europe and the USA. HIV-1 group M is composed of genetic subtypes (A, B, C, D, F, G, H, J, and K) and circulating recombinant forms (CRFs). HIV-1 subtype B is the predominant subtype in the USA, Europe, Japan, and Australia, but a significant percentage of new HIV-1 infections in Europe is caused by non-B subtypes.

A closely related, but distinct type of immunodeficiency virus is designated human immunodeficiency virus type 2 (HIV-2). Human immunodeficiency virus type 2 is similar to HIV-1 in its structural morphology, genomic organization, cell tropism, in vitro cytopathogenicity, transmission routes, and ability to cause AIDS. HIV-2 is less pathogenic than HIV-1, and HIV-2 infections have a longer latency period with slower progression to full-blown disease, lower viral titres, and lower rates of vertical and horizontal transmission. HIV-2 is endemic to West Africa, but HIV-2 infections, at a lower frequency compared to HIV-1, have been identified in the USA, Europe, Asia, and other regions of Africa.

Serological cross-reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample. This variability requires the inclusion of antigens to both HIV-1 and HIV-2 for the screening of antibodies to HIV-1 and HIV-2. The presence of HIV-1 and/or HIV-2 antibodies and/or p24 antigen in the blood indicates potential infection with HIV-1 and/or HIV-2. Early after infection with HIV, but prior to seroconversion, HIV antigens may be detected in serum or plasma specimens. The HIV structural protein most often used as the marker of antigenaemia is the core protein, p24. The LIAISON® XL murex HIV Ab / Ag HT assay uses HIV p24 monoclonal antibodies to detect HIV p24 antigen prior to seroconversion, thereby decreasing the seroconversion window and improving early detection of HIV infection.

3. PRINCIPLE OF THE PROCEDURE

The assay uses one reagent integral for the simultaneous detection of anti-HIV and HIV p24 antigen. The method for qualitative determination of specific antibodies to HIV and HIV p24 antigen is a *sandwich* chemiluminescence immunoassay (CLIA). HIV-1 recombinant antigen, HIV-1 group O and HIV-2 biotinylated peptides, and biotinylated monoclonal antibodies anti-HIV p24 antigen are used for coating magnetic particles (solid phase) and are also linked to isoluminol or fluorescein derivatives (isoluminol-antigen-peptides-monoclonal conjugates and monoclonal anti-HIV p24-Fluorescein conjugates). During the first incubation, HIV antibodies present in samples or controls and HIV p24 antigen present in calibrator, samples or controls bind to the solid phase and, for HIV p24 antigen, to monoclonals anti-HIV p24 antigen labelled with fluorescein. During the second incubation, HIV-1 antigen, HIV-1 group O, HIV-2 peptides, monoclonal anti-HIV p24 antigen and monoclonal anti-fluorescein linked to an isoluminol derivative (isoluminol-antigen conjugate) react with HIV antibodies, HIV p24 antigen and monoclonals anti-HIV p24 antigen labelled with fluorescein 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-antigen/peptide/monoclonal conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of anti-HIV-1/2/O antibodies or HIV p24 antigen presence in calibrator, samples or controls.

4. MATERIALS PROVIDED

Reagent integral for anti-HIV antibodies and HIV p24 antigen detection

Magnetic particles (2.5 mL)	SORB	Magnetic particles coated with HIV-1 gp41 (group M) recombinant antigen obtained in *E. coli,* HIV-1 group O and HIV-2 biotinylated peptides, biotinylated monoclonals anti-HIV p24 antigen BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (2.9 mL)	CAL	Low levels of HIV p24 recombinant antigen (obtained in E. coli), bovine aprotinin, casein, PBS buffer, 0.2% ProClin™ 300, an inert yellow dye.
Assay Buffer 1 (10.8 mL)	BUF 1	Monoclonals anti-HIV p24 antigen conjugated with fluorescein, bovine serum, casein, BSA, non-specific IgG (mouse polyclonal), HEPES buffer, detergent, EDTA, 0.2% ProClin™ 300, preservatives.
Conjugate (2X23 mL)	CONJ	HIV-1 gp41 (group M) recombinant antigen (obtained in *E. coli*), HIV-1 group O, HIV-2 peptides, monoclonals anti-HIV p24 antigen and monoclonal anti-fluorescein, conjugated to an isoluminol derivative, sheep serum, negative human serum, casein, non-specific IgG (mouse polyclonal), BSA, TRIS buffer, 0.2% ProClin™ 300, <11% Ethylene glycol, preservatives.
Number of tests		200

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

Materials required but not provided

LIAISON® XL 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 HIV Ab / Ag HT Controls (negative, positive for antibodies anti-HIV-1 and positive for HIV p24 antigen) (REF 310291).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

All serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, and anti-HIV-2 and found to be non-reactive, except for the positive control for anti-HIV-1, which is reactive for HIV-1 antibodies. The units positive for HIV antibodies have been inactivated by heat treatment (56°C for one hour) during the manufacturing process. They may derive from patients infected with HIV-1 and/or HIV-2 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 during the assay.

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 infec-tious 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), hazardous reagents are classified and labeled as follows:

REAGENTS:	CAL, BUF 1	CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 STOT RE 2 H373 Aquatic chronic 3 H412
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark	  GHS07 Exclamation mark GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H317 May cause an allergic skin reaction. H373 May cause damage to organs (kidney) through prolonged or repeated exposure. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/ vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.	P261 Avoid breathing dust/fume/gas/mist/vapours/ spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse. P314 Get medical advice/attention if you feel unwell.
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).	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); Ethylene glycol.

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

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

7. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

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

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. 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

- 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.

CONTROLS

Refer to the LIAISON® XL murex HIV Ab / Ag HT 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.
- **Storage and Handling**: Protect from sunlight. 
  Protect reagent packs from all heat and light sources.
- **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.

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), CPD (citrate-phosphate-dextrose), CPDA (citrate-phosphate-dextrose-adenine) have been tested and may be used with this assay. The correct specimen type must be used in the assay. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Follow manufacturers' instructions carefully when using plasma collection containers and gel separator containers. Samples having particulate matter, turbidity, lipaemia, or erythrocyte debris and specimens that have been thawed may require clarification by filtration or centrifugation before testing **because they could give interference and false Reactive 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. If the assay is performed within seven 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. Thirty samples with different reactivity were stored for seven days at 2-8°C and ten samples underwent **six** freeze-thaw cycles. The results showed no significant differences; however multiple freeze-thaw cycles should be avoided. The minimum volume required for determination is 350 µL specimen (200 µL specimen + 150 µL dead volume).

10. CALIBRATION

Assay of calibrator contained in the specific reagent integral allows the analyzer to set the cut-off of each detection assay. Each calibrator solution allows four calibrations to be performed.

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

– A new lot of Starter Kit is used.
– The previous calibration was performed more than four weeks before.
– Each time a new lot of 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.

Assay procedure for anti-HIV antibodies and HIV p24 antigen detection

The analyzer operations are as follows:

1. Dispense calibrator, controls or specimens into the reaction cuvettes.
2. Dispense coated magnetic particles.
3. Dispense Assay Buffer.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense Conjugate into the reaction cuvettes.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Reagents and measure the light emitted.

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 HIV Ab / Ag HT controls
(a) at least once per day of use,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
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 HIV p24 antigen and/or HIV 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-cut-off (S/CO) ratios for each test, and then grades the final results. For details, refer to the analyzer operator's manual.

Sample results should be interpreted as follows:

Specimens with signal-to-cut-off (S/CO) ratios below 1.00 are considered *non-reactive* for HIV p24 antigen and/or anti-HIV antibodies.

Specimens with signal-to-cut-off (S/CO) ratios above or equal to 1.00 are considered *reactive* for HIV p24 antigen and/or anti-HIV antibodies.

Specimens that show an initially reactive result must be centrifuged and retested in duplicate. Repeated reactivity is highly predictive of the presence of HIV p24 antigen and/or anti-HIV antibodies. However, like all immunoassays, the LIAISON® XL murex HIV Ab / Ag HT assay may occasionally yield non-specific reactions due to other causes. A repeatedly reactive specimen should be investigated further with sensitive, supplemental HIV-specific tests, such as immunoblot and antigen tests as well as HIV nucleic acid tests.

Retest algorithm for final interpretation of results.

Initial result	Action required	Retest result	Final interpretation
Reactive.	Retest in duplicate.	Both results are non-reactive.	Negative.
Reactive.	Retest in duplicate.	One or both retest results are reactive.	Positive, needs supplemental assay.
Non-Reactive.	No retest required.	–	Negative.

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 HIV p24 antigen and/or HIV antibodies does NOT exclude the possibility of exposure to or infection with HIV. In fact, either the patient may be unable to synthesize HIV specific antibodies or the circulating levels of p24 antigen and/or HIV specific antibodies may be below the assay detection limit.

In treated subjects the monitoring of HIV RNA levels is recommended to understand how well therapy is working, while serological testing on HIV-1 p24 antigen or anti-HIV antibodies provides poor diagnostic value.

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 HIV prevalence in the population being screened.

A reactive test result, likewise, does NOT constitute a diagnosis of AIDS, but may be indicative of recent and/or past HIV infection. Diagnosis of AIDS and AIDS-related diseases can be established only based on clinical evidence. 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 AIDS and related clinical conditions includes examination of the patient's immune status and clinical history.

Antibodies to HIV may be present after participation in an HIV vaccine study. Interpretation of this diagnostic result depends on the type of vaccine administered.

If LIAISON® XL MUREX HIV Ab / Ag HT results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

Results obtained with LIAISON® XL MUREX HIV Ab / Ag HT assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

Specimens from patients receiving therapeutic doses of vitamin H (biotin) theoretically may interfere in immunoassays based on biotinylated reagents but no interference from biotin was observed in LIAISON® XL murex HIV Ab / Ag HT assay up to 500 ng/mL.

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, CPD, CPDA), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin) or by a limited number of freeze-thaw cycles of samples. Results are not influenced by the use of same-day fresh samples positive for both analytes (anti-HIV antibodies and/or HIV p24 antigen) as a comparative study in 25 freshly collected specimens demonstrates.

Cross-reactions. The cross-reactivity study for the LIAISON® XL murex HIV Ab / Ag HT 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-1, HSV-2, Measles, VZV, HAV, HBV, HCV, HTLV-I/II, A/B Flu) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, human anti-mouse antibodies, anti-*E.coli*). The presence of potential cross-reactants in the samples was detected using CE-marked assays.

Condition	Number of expected negative samples	LIAISON® XL positive results
Parvovirus B19	16	0
Rubella virus	11	0
VZV	22	0
EBV IgM	9	0
EBV (VCA) IgG	12	0
Toxoplasma gondii	17	0
Borrelia burgdorferi	14	0
CMV	20	0
Anti-nuclear Autoantibodies (ANA)	11	0
HAV	17	0
HCV	12	0
HBsAg	13	0
Anti-HBc	17	0
Syphilis	30	0
HTLV I/II	30	1
A/B Flu	12	0
Measles	5	0
Anti-*E.coli*	5	0
HSV 1/2	14	0
HSV 2	5	0
HAMA	15	0
Rheumatoid factor	36	0
Total	343	1

15.2. Analytical sensitivity

The study performed with the WHO First International Reference Reagent for HIV-1 p24 antigen, NIBSC code: 90/636, showed a sensitivity of **0.59** IU/mL.

15.3. Precision for anti-HIV antibodies and HIV p24 antigen detection

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	C	D	E	F	G	H	CN	CP Ab	CP Ag
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.336	0.544	1.39	3.03	1.53	1.64	1.48	2.93	0.459	2.06	2.82
Standard deviation (S/CO)	0.016	0.058	0.049	0.209	0.083	0.090	0.070	0.155	0.027	0.096	0.150
Coefficient of variation (%)	4.9	10.6	3.5	6.9	5.5	5.5	4.7	5.3	6.0	4.7	5.3
Min. value (S/CO)	0.303	0.461	1.25	2.55	1.36	1.43	1.32	2.56	0.381	1.89	2.29
Max. value (S/CO)	0.367	0.651	1.45	3.27	1.63	1.81	1.62	3.10	0.494	2.21	3.04

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 in two different sites.

Reproducibility - Site 1	A	B	C	D	E	F	G	H	CN	CP Ab	CP Ag
LOT No. 01											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.392	0.458	1.58	1.65	1.50	3.37	1.70	2.85	0.370	2.14	2.69
Standard deviation (S/CO)	0.063	0.072	0.078	0.089	0.060	0.179	0.068	0.150	0.046	0.115	0.156
Coefficient of variation (%)	16.1	15.6	4.9	5.4	4.0	5.3	4.0	5.3	12.5	5.4	5.8
Min. value (S/CO)	0.314	0.322	1.44	1.46	1.37	3.07	1.58	2.56	0.309	1.99	2.37
Max. value (S/CO)	0.543	0.631	1.75	1.83	1.61	3.79	1.86	3.11	0.444	2.36	2.98
LOT No. 02											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.366	0.458	1.80	1.69	1.36	3.87	1.64	2.57	0.359	2.41	2.48
Standard deviation (S/CO)	0.044	0.072	0.097	0.093	0.065	0.238	0.107	0.125	0.044	0.130	0.112
Coefficient of variation (%)	12.1	15.8	5.4	5.5	4.8	6.1	6.5	4.9	12.3	5.4	4.5
Min. value (S/CO)	0.301	0.301	1.62	1.50	1.25	3.50	1.43	2.34	0.292	2.15	2.30
Max. value (S/CO)	0.451	0.580	2.01	1.86	1.46	4.33	1.81	2.82	0.442	2.60	2.68
LOT No. 03											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.382	0.454	1.71	1.64	1.45	3.77	1.63	2.79	0.373	2.30	2.64
Standard deviation (S/CO)	0.058	0.076	0.121	0.092	0.071	0.178	0.073	0.112	0.060	0.161	0.191
Coefficient of variation (%)	15.1	16.8	7.1	5.6	4.9	4.7	4.5	4.0	16.1	7.0	7.2
Min. value (S/CO)	0.298	0.294	1.51	1.50	1.32	3.37	1.50	2.58	0.307	1.83	2.36
Max. value (S/CO)	0.480	0.600	2.11	1.81	1.60	4.05	1.80	3.01	0.497	2.48	3.06
Inter-lot coeff. of variation (%)	3.5	0.5	6.4	1.7	5.3	7.1	2.2	5.4	2.0	5.8	4.2

Reproducibility - Site 2	A	B	C	D	E	F	G	H	CN	CP Ab	CP Ag
LOT No. 01											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.414	0.517	1.78	1.99	1.81	3.75	2.08	3.50	0.459	2.35	3.39
Standard deviation (S/CO)	0.071	0.067	0.118	0.140	0.129	0.153	0.115	0.254	0.095	0.180	0.157
Coefficient of variation (%)	17.2	12.9	6.6	7.1	7.1	4.1	5.5	7.2	20.6	7.7	4.6
Min. value (S/CO)	0.311	0.411	1.59	1.74	1.62	3.57	1.89	3.08	0.312	1.92	2.95
Max. value (S/CO)	0.515	0.655	2.11	2.25	2.08	4.28	2.32	3.91	0.589	2.64	3.62
LOT No. 02											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.371	0.432	1.66	1.67	1.43	3.53	1.59	2.70	0.377	2.12	2.61
Standard deviation (S/CO)	0.058	0.041	0.090	0.127	0.119	0.159	0.098	0.179	0.053	0.166	0.165
Coefficient of variation (%)	15.8	9.5	5.4	7.6	8.3	4.5	6.1	6.6	14.2	7.8	6.3
Min. value (S/CO)	0.287	0.376	1.52	1.46	1.24	3.23	1.45	2.43	0.297	1.88	2.36
Max. value (S/CO)	0.451	0.516	1.80	1.95	1.65	3.88	1.82	2.96	0.459	2.41	2.90
LOT No. 03											
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (S/CO)	0.428	0.525	1.81	1.78	1.71	3.88	1.77	3.37	0.433	2.30	3.19
Standard deviation (S/CO)	0.074	0.061	0.117	0.116	0.098	0.165	0.082	0.136	0.068	0.183	0.158
Coefficient of variation (%)	17.3	11.7	6.4	6.5	5.7	4.2	4.6	4.0	15.8	7.9	4.9
Min. value (S/CO)	0.322	0.422	1.58	1.60	1.53	3.62	1.62	3.09	0.339	1.94	2.82
Max. value (S/CO)	0.549	0.642	2.06	1.98	1.87	4.13	1.92	3.57	0.565	2.66	3.41
Inter-lot coeff. of variation (%)	7.4	10.5	4.8	8.8	12.2	4.7	13.7	13.5	10.0	5.3	13.3

15.4. High-dose saturation effect

Whenever samples containing extremely high antibody or antigen 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 five high-titred samples positive for anti-HIV antibodies or HIV p24 antigen. All samples resulted in very high signals that would be expected with high-titred samples, indicating no sample misclassification.

15.5. Reference to international standard

A study performed to evaluate the cut-off value for HIV p24 antigen of LIAISON® XL murex HIV Ab / Ag immunoassay demonstrated that it equates to **0.59** IU/mL WHO First International Reference Reagent (NIBSC code 90/636).
In addition, the cut-off value for HIV p24 antigen was evaluated by testing the HIV-1 Panel of the French Agency for Health Safety (Agence Française de Sécurité Sanitaire des Produits de Santé, Afssaps) with three lots of LIAISON® XL murex HIV Ab / Ag HT assay. The results show that the cut-off value equates to **11.12** pg/mL.

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 **5910** serum and plasma specimens collected in two blood donation centres (including **300** specimens from first-time donors). Specimens tested were expected negative samples from an unselected blood donor population with zero prevalence of HIV infection. The assay shows diagnostic specificity above 99.5%. Additional specimens were also tested, randomly selected from hospitalized/diagnostic 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	5910	11	6	99.90 (5904/5910)	99.78-99.96
Hospitalized/ diagnostic patients	2094	4	4	99.81 (2090/2094)	99.51-99.95
Dialysis patients	251	1	1	99.60 (250/251)	97.80-99.99
Pregnant women	1001	8	8	99.20 (993/1001)	98.43-99.66
High-risk subjects	471	3	1	99.79 (470/471)	98.83-100.0

16.2. Diagnostic sensitivity

Diagnostic sensitivity was assessed by testing 875 specimens from anti-HIV-1-positive patients (226 of whom with defined anti-HIV subtypes: 25 subtype A, 26 subtype B, 25 subtype C, 23 subtype D, 21 subtype F, 16 subtype G, 5 subtype H, 1 subtype J, 3 non-B, 62 CRF and 19 anti-HIV-1 group O), 302 samples from anti-HIV-2-positive patients as well as 146 samples from HIV p24-positive patients. Data of this study are summarized in Table II (95% CI = 95% confidence interval).

In an additional study the ability of the LIAISON® XL murex HIV Ab / Ag HT assay to detect anti-HIV antibodies and HIV p24 antigen was evaluated by testing sequentially-collected specimens belonging to 56 seroconversion panels from donors who seroconverted over the course of their donation history. Commercially available, precharacterized panels for anti-HIV antibodies and HIV p24 antigen were used, each starting with a negative bleed and exhibiting narrow bleeding intervals. The panels were also tested by a reference HIV Combo assay. The test diagnostic sensitivity in the detection of HIV early infection is substantially equivalent to or better than the reference assay.

Table II - Diagnostic sensitivity.

Population	Number of cases	Reactive samples, No.	Diagnostic sensitivity, %	Diagnostic sensitivity, 95% CI
Anti-HIV-1-positive patients	856	856	100.0 (856/856)	99.57-100.0
Anti-HIV-O-positive patients	19	19	100.0 (19/19)	82.35-100.0
Anti-HIV-2-positive patients	302	302	100.0 (302/302)	98.79-100.0
Total	1177	1177	100.0 (1177/1177)	99.69-100.0
HIV p24-positive patients	146	146	100.0 (146/146)	97.51-100.0

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