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

CE 0459

Changes: §1, §2, §4, §5, §6, §7, §8, §9, §10, §12, §15.2, §15.3, References;
Deletions: §14;

LIAISON® HSV-1/2 IgG (REF 310800)

1. INTENDED PURPOSE

The LIAISON® HSV-1/2 IgG assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro qualitative determination of specific IgG antibodies to Herpes simplex virus Types 1 and/or 2 (HSV-1 and/or HSV-2) in human serum or plasma samples. The assay is intended as an aid in the diagnosis of HSV-1 and/or HSV-2 infection.
The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Herpes simplex viruses (HSV) are doublestranded DNA viruses that belong to the Herpesviridae family and are divided into two serotypes, HSV-1 and HSV-2. Although they share molecular and biological characteristics, they have unique antigenic profiles as well as different epidemiology and clinical patterns.[1] The difference between types 1 and 2 is in the glycoprotein layer, specifically how the gG1 glycoprotein in HSV-1 differs from gG2 in HSV-2, a difference used for type-discriminatory serology.[3,4] HSV can exist in both latent and lytic states. In the latent period, infected cells may persist throughout the life of the host. In lytic infection, there is viral replication and viral spread causing infection of the skin surface and mucous membranes.[2,5] HSV-1 is most often transmitted during childhood and adolescence. Infection with HSV-1 is characterized by oral and facial lesions. Although HSV-1 is most often transmitted through nonsexual contact, studies indicate a significant increase in the number of HSV-1-associated genital herpes cases.[6,7] HSV-2 infection is the leading cause of genital herpes and is considered one of the most prevalent sexually transmitted diseases worldwide.[5,8] Primary HSV-2 infection during pregnancy has been linked to miscarriage, prematurity, congenital infection and neonatal herpes.[3] Mother-to-child infection occurs at the time of birth, when the newborn crosses the vaginal canal and comes into contact with the mother's lesions. Neonatal herpes can manifest as encephalitis with or without skin involvement or as a disseminated multi-organ infection, often fatal.[9,10] The International Union against Sexually Transmitted Infections (IUSTI, Europe) recommends HSV type-specific serological testing in patients with a history of recurrent or atypical genital disease when direct detection methods have returned negative.[11] IUSTI recommends also that asymptomatic pregnant women are routinely tested for HSV antibodies if the partner has a history of genital herpes. The World Health Organization (WHO) has identical recommendations.[8] The Centers for Disease Control and Prevention (CDC, United States) recommends type-specific HSV IgG antibody testing for individuals with recurrent genital symptoms or atypical symptoms with negative HSV PCR or culture results, for patients whose partner has genital herpes, for asymptomatic pregnant women at risk for an HSV infection, for persons undergoing an evaluation of sexually transmitted diseases, for human immunodeficiency virus (HIV)-infected patients or men who have sex with men at increased risk for HIV acquisition.[6] When an individual contracts herpes virus, the immune system responds by developing antibodies against the virus. IgM antibodies usually become detectable in the first 10 days after exposure to the virus and may remain detectable for up to six weeks in certain individuals.[12] Anti-HSV IgM antibody titers increase to four times the baseline value 2 to 4 weeks after infection.[13] Most genital HSV infections go unnoticed, therefore the detection of serum HSV-1 and -2 IgM antibodies helps in the diagnosis of infection. In IgG-negative patients, evaluation for IgM antibodies increases the likelihood of detecting an early infection. IgM antibodies can also be present during recurrent infection. Because IgM antibodies are not type specific, they do not allow to differentiate between HSV-1 and HSV-2 infections.[9,14,6] Both HSV-1 and HSV-2 induce a humoral immune response with the production of anti-HSV IgG and IgM antibodies. As a result, serological analysis can be used to diagnose an HSV-1 or HSV-2 infection. Serological testing, helps in detecting the infection, independent of the presence of clinical symptoms.[15,13] Likewise, IgM positivity in patients with sexually transmitted diseases may be due to serum conversion of primary infection or reactivation. Thus, serum HSV-1 and -2 IgM can be used for periodic screening in patients with sexually transmitted diseases, to follow HSV trends, transmissibility and viral load.[13]
Serological testing is recommended as an aid in the diagnosis of genital herpes in patients with recurrent genital symptoms, atypical lesions and negative HSV cultures.[16] Serological diagnosis can be used for asymptomatic individuals, pregnant women at risk of acquiring HSV infection near childbirth, men who have sex with men and people with the HIV virus.[17,18] Diagnosis is also recommended for children born to mothers who had genital herpes during pregnancy, individuals with a recurrent history of ulcerative disease[17], sexual partners of genital herpes patients[19] and males who have multiple sex partners.[20]

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of specific IgG to HSV is an indirect chemiluminescence immunoassay (CLIA). HSV recombinant proteins are used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, HSV antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with HSV IgG 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 HSV IgG concentration present in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥0.25% solid) coated with HSV recombinant proteins (approx. 50 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.5 mL)	CAL 1	Human serum/plasma containing low HSV IgG levels (approx. 1 index), BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations (index) are referenced to an in-house antibody preparation.
Calibrator 2 (2.5 mL)	CAL 2	Human serum/plasma containing high HSV IgG levels (approx. 18.5 index), BSA, phosphate buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (index) are referenced to an in-house antibody preparation.
Buffer C (2.3 mL)	BUF C	BSA, phosphate buffer, 0.2% ProClin® 300.
Specimen diluent (28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (23 mL)	CONJ	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate buffer, 0.2% ProClin® 300, preservatives.
Number of tests		100

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 (system related)

LIAISON® XL Analyzer	LIAISON® Analyzer
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055). – LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300). – LIAISON® Wash/System Liquid (REF 319100). LIAISON® XL Waste Bags (REF X0025). –	LIAISON® Module (REF 319130). – – LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300). LIAISON® Light Check 12 (REF 319150). LIAISON® Wash/System Liquid (REF 319100). LIAISON® Waste Bags (REF 450003). LIAISON® Cleaning Kit (REF 310990)

LIAISON® XS Analyzer	
LIAISON® Cuvettes on Tray (REF X0053). LIAISON® Disposable Tips (REF X0055). LIAISON® EASY Starter Kit (REF 319300). LIAISON® EASY Wash Buffer (REF 319301). LIAISON® EASY System Liquid (REF 319302). LIAISON® EASY Waste (REF X0054). LIAISON® EASY Cleaning Tool (REF 310996)	

Additionally required materials

LIAISON® HSV-1/2 IgG controls (negative and positive) (REF 310801).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use. For Laboratory Professional Use Only.

Visually inspect the integral vials for leaking at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

All serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

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.

The analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.

Do not use kits or components beyond the expiration date given on the label.

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

REAGENTS:	CAL 1, CAL 2, BUF C, DIL SPE, 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).

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

Incomplete magnetic particle 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, 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® Analyzer

- Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 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.

LIAISON® XL and LIAISON® XS analyzers

- LIAISON® XL and LIAISON® XS analyzers are 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.

8. STORAGE AND STABILITY OF REAGENT INTEGRAL

- **Sealed**: stable at 2-8°C until the expiry date.
- **Opened on board or at 2-8°C**: up to eight (8) weeks.
- Use the storage rack provided with the LIAISON® Analyzer family for upright storage of the reagent integral.
- Do not freeze.
- Keep upright for storage to facilitate subsequent proper resuspension of the magnetic particles.
- Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

The correct specimen type must be used in the assay. Following matrices have been tested and may be used:
– serum;
– heparin plasma;
– potassium EDTA plasma;
– citrate plasma.

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.

Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.

Package and label specimens in compliance with applicable regulations covering the transport of clinical specimens and infectious substances.

Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.

Uncontrolled transport conditions (in terms of temperature and time) may 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).

A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells or gel separator. The following storage conditions showed no significant differences:
– room temperature storage should be avoided;
– 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to 5 freeze-thaw cycles, however multiple freeze thaw cycles should be avoided.

If samples are stored frozen, mix thawed samples well before testing. Further centrifugation of specimens removed from red cells, clot or gel separator (suggested between 3,000 and 10,000 g for 10 minutes) is recommended to guarantee the consistency of results whenever one of the following conditions is identified:
– Samples previously centrifuged and stored at 2°-8°C;
– Samples with particulate matter, fibrin, turbidity, lipaemia or erythrocyte debris;
– Samples frozen and thawed;
– Samples requiring repeat testing.

Specimens with a lipid layer on the top should be transferred into 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. Heat inactivation of the specimens may affect the test results. Check for and remove air bubbles before assaying.

The minimum volume required for a single determination is 190 µL of specimen (40 µL specimen + 150 µL dead volume).

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 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 four (4) weeks before.
– **LIAISON® and LIAISON® XL analyzers**: the analyzer has been serviced.
– **LIAISON® XS analyzer**: after a technical intervention, only if required by the service procedure, as communicated by DiaSorin Technical support or representative.
– Control values lie outside the expected ranges.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).
LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label 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.

LIAISON® XL and LIAISON® XS analyzers. 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 coated magnetic particles.
3. Dispense buffer C.
4. Dispense specimen diluent.
5. Incubate.
6. Wash with Wash/System liquid.
7. Dispense conjugate into the reaction module.
8. Incubate.
9. Wash with Wash/System liquid.
10. Add the Starter Kit and measure the light emitted.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® HSV-1/2 IgG controls (REF 310801)
(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,
(e) to assess adequacy of performance of the open integral 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 analyzer automatically calculates HSV IgG levels expressed as index value and grades the results. For details, refer to the analyzer operator's manual.
Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

The cut-off value discriminating between the presence and the absence of HSV IgG has an index value of 1. Sample results should be interpreted as follows:
Samples with HSV IgG levels below an index value of 0.9 should be graded *negative*.
Samples with HSV IgG levels ranging between an index value of 0.9 and 1.1 should be graded *equivocal. Equivocal samples must be retested in order to confirm the initial result. Samples which are positive at the second test should be considered positive. Samples which are negative at the second test should be considered negative. A second sample should be collected and tested no less than one week later when the result is repeatedly equivocal.*
Samples with HSV IgG levels equal to or above an index value of 1.1 should be graded *positive*.

A negative result generally indicates that the patient has not been infected, but does not always rule out acute HSV infection. It should be underlined that the test scores negative during the first two to three weeks after infection. If clinical exposure to HSV is suspected despite a negative finding, a second sample should be collected and tested no less than one week later.
Seroconversion from a negative sample to a positive sample is evidence of either recent or in progress infection, or administration of HSV immunoglobulin. In recurrent HSV infection, significant titre changes are likely neither in HSV-1 nor in HSV-2 infection.
A positive result generally indicates exposure to the pathogen, or administration of HSV immunoglobulin.

Test results are reported qualitatively as positive or negative for the presence of HSV IgG. 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.

LIAISON® HSV-1/2 IgG assay is of value to assess the immunological status of sexually active adults or pregnant women towards Herpes simplex viruses. In conjunction with LIAISON® HSV-2 IgG assay, the test can be used to distinguish subjects infected by isolated HSV-1 from subjects infected by HSV-1 and/or HSV-2, as illustrated in the table herebelow.

HSV-1/2 IgG result	HSV-2 IgG result	Interpretation
negative	negative	Not exposed subjects, negative for HSV-1 and HSV-2 IgG.
positive	negative	Isolated HSV-1 infection.
positive	positive	Isolated HSV-2 infection or associated HSV-1 and HSV-2 infections.

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.
- Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.

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, EDTA, heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), or by freeze-thaw cycles of samples.

Cross-reactions. As a rule, the presence of potentially cross-reactive antibodies does not interfere in the assay. The antibodies investigated were: *(a)* immunoglobulins to various infectious agents – such as hCMV, EBV, VZV, rubella virus, *Toxoplasma gondii* – *(b)* rheumatoid factor (anti-Fc immunoglobulin) antibodies.

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

Repeatability	A	B	C	D
Number of determinations	20	20	20	20
Mean (index value)	0.16	0.26	5.10	9.70
Standard deviation	0.023	0.006	0.163	0.233
Coefficient of variation (%)	14.6	2.3	3.2	2.4

Reproducibility	B	A	C	D
Number of determinations	20	20	20	20
Mean (index value)	0.14	0.26	3.70	6.10
Standard deviation	0.014	0.018	0.170	0.329
Coefficient of variation (%)	10.2	7.0	4.6	5.4

Lot-to-Lot Reproducibility. Six samples tested in singleton on five different LIAISON® instruments on four different batches.

Reproducibility	LIAISON® HSV 1-2 IgG (Code 310800) on LIAISON® Analyzer					
Sample ID	E	F	G*	H	Negative Control*	Positive Control
Mean (Index)	7.72	4.50	13841	14.38	5805	8.69
Inter-lot coefficient of variation (%)	5.1	8.5	22.2	6.6	22.2	6.2

*RLU values

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

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

Repeatability	1	2	3	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.423	2.06	8.10	10.2
Standard deviation	0.021	0.076	0.30	0.28
Coefficient of variation (%)	4.9	3.7	3.7	2.8
Min. value (index value)	0.380	1.93	7.49	9.87
Max. value (index value)	0.458	2.26	8.53	10.8

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) to evaluate reproducibility.

Reproducibility	1	2	3	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.437	2.00	7.69	9.88
Standard deviation	0.072	0.11	0.63	0.32
Coefficient of variation (%)	16.4	5.5	8.2	3.3
Min. value (index value)	0.362	1.70	5.47	9.36
Max. value (index value)	0.647	2.19	8.42	10.5

Lot-to-Lot Reproducibility. Six samples tested in singleton on five different LIAISON® XL instruments on four different batches.

Reproducibility	LIAISON® HSV 1-2 IgG (Code 310800) on LIAISON® XL Analyzer					
Sample ID	10	11	12*	13	Negative Control*	Positive Control
Mean (Index)	8.87	4.98	57138	16.36	14802	10.02
Inter-lot coefficient of variation (%)	8.8	4.0	0.9	11.4	17.4	6.7

*RLU values

15.4. Precision with LIAISON® XS Analyzer

A five day precision study was conducted on three LIAISON® XS Analyzers to verify the precision with the LIAISON® HSV-1/2 IgG Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of seven frozen samples was used for the study.

The samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels. The LIAISON® Control HSV-1/2 IgG set was also included in the five day study.

The coded panel was tested on three LIAISON® XS Analyzers, in six replicates in a single run per day, for 5 operative days.

The mean Index value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same run to evaluate repeatability.

Repeatability	4	5	6	7	8	9	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (index value)	0.920	1.98	1.80	3.64	5.59	16.8	8.99
Standard deviation	0.022	0.036	0.039	0.104	0.142	0.668	0.348
Coefficient of variation (%)	2.4	1.8	2.2	2.9	2.5	3.6	3.9
Min. value (index value)	0.721	1.84	1.63	3.01	4.87	11.6	6.84
Max. value (index value)	1.01	2.11	2.00	3.96	6.16	18.4	10.4

Reproducibility. Ninety replicates were performed in different days (one per day) to evaluate reproducibility.

Reproducibility	4	5	6	7	8	9	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (index value)	0.920	1.98	1.80	3.64	5.59	16.8	8.99
Standard deviation	0.070	0.056	0.061	0.142	0.189	0.813	0.444
Coefficient of variation (%)	7.6	2.8	3.4	3.9	3.4	4.8	4.9
Min. value (index value)	0.721	1.84	1.63	3.01	4.87	11.6	6.84
Max. value (index value)	1.01	2.11	2.00	3.96	6.16	18.4	10.4

15.5. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 268 specimens from different selected populations (subjects never infected by HSV-1 and HSV-2; patients affected by other infectious diseases with similar symptomatology; subjects positive for HSV-1 IgG and negative for HSV-2 IgG; subjects negative for HSV-1 IgG and positive for HSV-2 IgG; subjects positive for HSV-1 and HSV-2 IgG). The specimens were tested by several comparison methods and consensus between them as well as the available clinical and serological data were applied to define the expected results.

2 positive, one equivocal and 92 negative results were observed in the expected negative population studied - diagnostic specificity: 96.84% (95% confidence interval: 91.05-99.34%).

One negative and 172 positive results were observed in the expected positive population studied - diagnostic sensitivity: 99.42% (95% confidence interval: 96.82-99.99%).

A Summary of safety and performance is available on EUDAMED.

For EU only: please be aware that any serious incident that has occurred in relation to this IVD medical device should be reported to DiaSorin Italia S.p.A. and to the Competent Authority of the EU Member State in which the user and/or the patient is established.

REFERENCES

1 Jun, W. et al. Expression and characterization of the soluble form of recombinant mature HSV-2 glycoprotein G for use in anti-HSV-2 IgG serodiagnostic immunoassay. J Virol Methods 252, 65-69, (2018).

2 Lorenz, B. D. Herpes simplex virus infection. BMJ Best Practice, (2019).

3 Patel, E. U. et al. Precision of the Kalon Herpes Simplex Virus Type 2 IgG ELISA: an international inter-laboratory assessment. BMC Infect Dis 15, 398, (2015).

4 van Rooijen, M. S., Roest, W., hansen, G., Kwa, D. & de Vries, H. J. False-negative type-specific glycoprotein G antibody responses in STI clinic patients with recurrent HSV-1 or HSV-2 DNA positive genital herpes, The Netherlands. Sex Transm Infect 92, 257-260, (2016).

5 Domeika M, B. M., Savicheva A, Kolomiec N, Sokolovskiy E, Hallén A, Unemo M, Ballard RC. Guidelines for the laboratory diagnosis of genital herpes in eastern European countries. Euro Surveil4 (2010).

6 CDC. Sexually Transmitted Diseases Treatment Guidelines. Morbidity and Mortality Weekly Report,Recommendations and Reports 64, (2015).

7 Debrah, O. et al. SERO-prevalence of herpes simplex virus type 1 and type 2 among women attending routine Cervicare clinics in Ghana. BMCInfect Dis 18, 378, (2018).

8 WHO. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. World Health Organization 2013, (2013).

9 Bikup, U. G., et al. Laboratory diagnosis and epidemiology of herpes simplex 1 and 2 genital infections. Acta Dermatovenero! APA, (2015).

10 Tavakoli, A. et al. Asymptomatic Herpes Simplex Virus Infection in Iranian Mothers and Their Newborns. Feta! Pediatr Pathol 36, 27-32, (2017)

11. Patel, R., Kennedy, O. .J , Emily Clarke, et al. 2017 European guidelines for the management of genital herpes. International Journal of STD & AIDS, (2017).

12 Amudha, V. P., Rashetha, SucilAthAngam,G. ,Cinthujah, B. Serological Profile of HSV 2 in STD Patients: Evaluation of Diagnostic Utility of HSV-2 IgM and IgG Detection. Journal of Clinical and Diagnostic Research., (2014).

13 Tada, D. G. a. N. K. Serum HSV-1 and 2 IgM in Sexually Transmitted Diseases - More for Screening Less for Diagnosis: An Evaluation of Clinical Manifestation. J Glob Infect Dis.., (2012).

14 LeGoff., e. a. Diagnosis of genital herpes simplex virus infection in the clinica! laboratory. VirologyJournal, (2014).

15 Okonko, I. O., Cookey, T. I., Okerentugba, P. O. & Frank-Peterside, N. Serum HSV1 and -2 IgM in pregnant women in Port Harcourt, Nigeria. J Immunoassay Immunochem 36, 343-358, (2015).

16 Vilibic-Cavlek, T., Kolaric, B., Bogdanic, M., Tabain, I. & Beader, N. Herpes Group Viruses: a Seroprevalence Study in Hemodialysis Patients.
Acta Clln Croat 56, 255-261, (2017).

17 Badawi, H. et al. Herpes simplex virus type-2 in Egyptian patients with biadder cancer or cystitis. Apmis 118, 37-44, (2010).

18 World Health Organization. Herpes simplex virus, <https://www.who.int/news room/fact-sheets/detail/herpes-simplex-virus> (2017).

19 Motamedifar, M., Sarvari, J., Ebrahimpour, A. & Emami, A. Symptomatic Reactivation of HSV Enfection Correlates with Decreased Serum Levels of TNF-alpha. Iran Immunol 12, 27-34, (2015).

20 J Page, J. T., R L Tideman, C Seifert, C Marks, A Cunningham, A Mindel.
Is HSV serology useful for the management of first episode genital herpes Sex Transm Infect, (2003).

21. BOWEN et al.,
Impact of blood collection devices on clinical chemistry assay
Clinical Biochemistry, 43, 4-25, 2010.

22. Clinical and Laboratory Standards Institute (CLSI) C15-A3, Vol.34, No.12,
User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition

200/007-835, 11 - 2024-05