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

Changes: §4, §6;
Deletions: -

LIAISON® HSV-1/2 IgM (REF 310820)

1. INTENDED USE

The LIAISON® HSV-1/2 IgM assay uses chemiluminescence immunoassay (CLIA) technology for the qualitative determination of specific IgM antibodies to Herpes simplex virus Types 1 and/or 2 (HSV-1 and/or HSV-2) in human serum or plasma samples. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Herpes simplex virus (HSV) is an enveloped, DNA-containing virus morphologically similar to the other members of the *Herpetoviridae* family. Two naturally occurring variants of HSV, with different biologic and epidemiologic characteristics, are recognized by restriction-endonuclease or antigenic analysis. Both types of virus cause infections in humans which range in severity from cold sores to encephalitis. HSV Type 1 (HSV-1) generally infects the mucous membrane of the eye, mouth and mucocutaneous junctions of the face, and is also one of the most common causes of severe sporadic encephalitis in adults. HSV Type 2 (HSV-2) is usually associated with mucocutaneous genital lesions: genital herpes is now one of the most common sexually transmitted diseases. The association between the site of infection and the HSV type involved is not, however, exclusive.

Once infection occurs, HSV persists in a latent state in sensory ganglia from where it may re-emerge to cause periodic recurrence of infection induced by many stimuli, which may or may not result in clinical lesions. Immunocompromized patients are more likely to have frequent HSV recurrences. This suggests that serum antibody and virus-specific cell-mediated immunity contribute to recovery.

Pregnant women who develop genital herpes are two to three times more likely to have spontaneous abortions or deliver a premature infant than are pregnant non-infected women. Active virus excretion in genital secretions of pregnant women may result in severe neonatal HSV infection contracted when the infant passes through an infected genital tract. When HSV lesions are present during delivery, 40% to 60% of the neonates can be affected. Transmission of HSV infection to neonates is associated with high morbidity and mortality rates if untreated.

By five years of age, 35% of children have antibody to HSV-1 and 80% of adults by age 25 will have specific antibodies to HSV-1. Since HSV-1 and HSV-2 share common antigenic determinants, antibody directed against one viral type may cross-react with the other viral type. Recurrent infections often occur with both viral types despite the presence of circulating antiviral antibodies.

Rapid and accurate diagnosis of HSV infection is necessary to ensure early implementation of selective antiviral chemotherapy and to minimize spread of infection. The first humoral immune response to infection is the synthesis of specific anti-HSV IgM antibody which becomes detectable one week after infection. Normally this is a proof of recent or recurrent infection. Specific IgG antibody generally appears two to three weeks after primary infection, but may fall in titre after a few months. Patients with recurring disease often do not show an increase in titre. Detection of IgG allows assessment of the patient's immune status and provides serological evidence of prior exposure to HSV. This may aid in the diagnosis of recent (primary or recurrent) HSV infection in paired sera by the presence of seroconversion to HSV-1 or HSV-2 antibody.

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of specific IgM to HSV is an indirect chemiluminescence immunoassay (CLIA). HSV viral lysate enriched with recombinant proteins is 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 IgM 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 IgM concentration present in calibrators, samples or controls. Buffer A contains goat IgG to human IgG as an absorbent reagent to curb interference from human IgG specific to HSV or from rheumatoid factor.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles coated with HSV viral lysate enriched with recombinant proteins, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (0.85 mL)	CAL 1	Human serum/plasma containing low HSV IgM levels, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye.
Calibrator 2 (0.85 mL)	CAL 2	Human serum/plasma containing high HSV IgM levels, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye.
Buffer A (25 mL)	BUF A	Goat IgG to human IgG (absorbent reagent), goat serum, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye.
Conjugate (23 mL)	CONJ	Mouse monoclonal antibodies to human IgM conjugated to an isoluminol derivative, non-specific IgG (mouse polyclonal), 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® XL Cleaning Tool (REF 310995) or LIAISON® EASY Cleaning Tool (REF 310996)	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 IgM controls (negative and positive) (REF 310821).

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, 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.

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 A, CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash 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.

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 Analyzer and LIAISON® XS Analyzer 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

Upon receipt, the reagent integral must be stored in an upright position to facilitate resuspension of magnetic particles. When the reagent integral is stored sealed and kept upright, the reagents are stable at 2-8°C up to the expiry date. Do not freeze. The reagent integral should not be used past the expiry date indicated on the kit and reagent integral labels. After removing the seals, the reagent integral is stable for eight weeks refrigerated either at 2-8°C or on board the instrument.

9. SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma may be used. The anticoagulants citrate, EDTA and heparin have been tested and may be used with this assay. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity, lipaemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. 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. Nine 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. The minimum volume required is 170 µL specimen (20 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 eight 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.

LIAISON® Analyzer: Calibrator values are stored in the reagent integral bar codes.

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. Dilute samples with buffer A
2. Dispense calibrators, controls or specimens into the reaction module.
3. Dispense buffer A.
4. Dispense coated magnetic particles.
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.

Warning - *Maintenance with the LIAISON® XL Cleaning Tool (REF 310995) or LIAISON® EASY Cleaning Tool (REF 310996) must be performed (refer to pertinent instruction for use for details).*

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 IgM 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,
(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 IgM 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 IgM has an index value of 1. Sample results should be interpreted as follows:
Samples with HSV IgM levels below an index value of 0.9 should be graded *negative*.
Samples with HSV IgM 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 IgM levels equal to or above an index value of 1.1 should be graded *positive*.

A positive result is indicative of recent infection. A negative result, however, does not always rule out acute HSV infection, because the infection may be in its very early stage, and the patient may still be unable to synthesize HSV-specific IgM. 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.

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

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.

HSV IgM antibodies may persist over a long period and a specific IgM response can be observed after reinfection, endogenous latent reactivation, or even during the course of other viral illnesses.

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. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON®, LIAISON® XL or LIAISON® XS).

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. The cross-reactivity study for the LIAISON® HSV-1/2 IgM assay was designed to evaluate potential interference from antibodies to other organisms that may cause infectious diseases (hCMV, parvovirus B19, *Toxoplasma gondii*, HBV, HAV, *Treponema pallidum*, VZV, measles virus, mumps virus, *Borrelia burgdorferi*, influenza viruses) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor). Samples for these studies were pre-screened with another commercially available HSV IgM assay. If found negative for HSV IgM 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.

Condition	Number of expected negative samples	LIAISON® positive or equivocal results
Parvovirus B19 IgM antibodies	52	4
Toxoplasma gondii IgM antibodies	12	1
HAV IgM antibodies	56	4
HBc IgM antibodies	6	2
hCMV IgM antibodies	14	2
Influenza viruses antibodies	8	0
Borrelia burgdorferi IgM antibodies	6	1
Mumps virus IgM antibodies	8	1
Measles virus IgM antibodies	2	0
VZV IgM antibodies	42	1
Treponema pallidum IgM antibodies	3	0
Anti-nuclear autoantibodies (ANA)	7	0
Rheumatoid factor (anti-Fc immunoglobulin)	10	0
Total	226	16

Assay interference may occur due to the presence of patient's IgM during early acute infection. Long-lasting IgM does not interfere in LIAISON® HSV-1/2 IgM assay.
Reactivity may be related to cross-reactions with early IgM to other viruses or to reappearance of HSV IgM as a consequence of polyclonal activation induced by other agents.

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

Repeatability	A	B	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.576	1.92	0.0631	2.86
Standard deviation	0.061	0.17	0.021	0.32
Coefficient of variation (%)	10.6	8.6	33.5	11.3
Min. value	0.474	1.58	0.0215	2.36
Max. value	0.714	2.17	0.0917	3.52

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

Reproducibility	A	B	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.625	1.93	0.0733	2.65
Standard deviation	0.096	0.25	0.038	0.36
Coefficient of variation (%)	15.3	13.0	51.4	13.8
Min. value	0.473	1.44	0.000	2.04
Max. value	0.778	2.31	0.132	3.19

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	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.654	1.80	0.0664	1.72
Standard deviation	0.023	0.15	0.0096	0.068
Coefficient of variation (%)	3.5	8.1	14.5	3.9
Min. value	0.601	1.67	0.0553	1.63
Max. value	0.696	2.27	0.0955	1.90

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

Reproducibility	1	2	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.581	1.67	0.0487	1.70
Standard deviation	0.11	0.22	0.017	0.12
Coefficient of variation (%)	19.3	13.5	34.3	6.8
Min. value	0.221	1.18	0.0118	1.50
Max. value	0.730	2.06	0.0750	1.93

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 IgM Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 7 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 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 test to evaluate repeatability. Seven serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Repeatability	3	4	5	6	7	8	9	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.792	0.901	1.46	1.63	1.55	1.86	2.44	1444	1.93
Standard deviation	0.034	0.031	0.041	0.068	0.042	0.075	0.074	46.2	0.041
Coefficient of variation (%)	4.3	3.5	2.8	4.2	2.7	4.0	3.0	3.2	2.1
Min. value	0.648	0.737	1.22	1.29	1.26	1.56	2.00	1243	1.66
Max. value	1.02	1.16	1.85	2.08	1.89	2.19	3.01	1646	2.30

*Negative Control is expressed in RLU because out of the Assay Range

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. Seven serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Reproducibility	3	4	5	6	7	8	9	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.792	0.901	1.46	1.63	1.55	1.86	2.44	1444	1.93
Standard deviation	0.048	0.050	0.079	0.107	0.083	0.134	0.181	88.876	0.112
Coefficient of variation (%)	6.0	5.5	5.4	6.6	5.4	7.2	7.4	6.2	5.8
Min. value	0.648	0.737	1.22	1.29	1.26	1.56	2.00	1243	1.66
Max. value	1.02	1.16	1.85	2.08	1.89	2.19	3.01	1646	2.30

*Negative Control is expressed in RLU because out of the Assay Range

15.5. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 233 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 IgM). 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. 29 specimens were unresolved by the reference methods and therefore were not included in the data analysis.

7 equivocal and 191 negative results were observed in the expected negative population studied - diagnostic specificity: 96.46% (95% confidence interval: 92.85-98.57%).

No negative and 6 positive results were observed in the expected positive population studied - diagnostic sensitivity: 100% (95% confidence interval: 54.07-100%).

REFERENCES

I. ALEXANDER, C.R. ASHLEY, K.J. SMITH, J. HARBOUR, A.P. ROOME, J.M. DARVILLE
Comparison of ELISA with virus isolation for the diagnosis of genital herpes.
J. Clin. Pathol., **38** : 554-557 (1985).

R.L. ASHLEY, A. WALD
Genital herpes: review of the epidemic and potential use of type-specific serology.
Clin. Microbiol. Rev., **12** (1) : 1-8 (1999).

R.L. ASHLEY
Sorting out the new HSV type-specific antibody tests.
Sex. Transm. Inf., **77** : 232-237 (2001).

Z.A. BROWN, L.A. VONTVER, J. BENEDETTI, C.W. CRITCHLOW, D.E. HACKOK, C.J. SELLS. S. BERRY, L. COREY
Genital herpes in pregnancy: risk factors associated with recurrences and asymptomatic viral shedding.
Am. J. Obstet. Gynecol., **153** : 24-30 (1985).

L. COREY
Laboratory diagnosis of Herpes simplex virus infections. Principles guiding the development of rapid diagnostic tests.
Diagn. Microbiol. Infect. Dis., **4** (Suppl. 3) : 1118-1198 (1986).

B.D. EING et al.
Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2.
J. Clin. Microbiol., **40** (2) : 407-413 (2002).

B. FRAME, J.B. MAHONY, N. BALACHANDRAN, W.E. RAWIS, M.A. CHERNENSKY
Identification and typing of Herpes simplex virus by enzyme immunoassay with monoclonal antibodies.
J. Clin. Microbiol., **20** : 162-166 (1984).

L. PEREIRA, R.M. COLEMAN, P.D. BAILEY, D. DONDERO, C. WICKLIFFE, A.J. NAHMIAS
Determination of Herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay.
J. Clin. Microbiol., **18** : 287-291 (1983).

R.J. WITHLEY, A.M. ARVIN
Herpes simplex virus infections.
In: Infectious Diseases of the Fetus and Newborn Infant, J.S. Remington, J.O. Klein eds., W.B. Saunders Co., p. 354-376, 1995.

M.G. REVELLO, R. GUALANDRI, R. MANSERVIGI, G. GERNA
Development and evaluation of an ELISA using secreted recombinant glycoprotein B for determination of IgG antibody to herpes simplex virus.
J. Virol. Meth., **34** : 57-70 (1991).

P. WUTZLER et al.
Seroprevalence of herpes simplex virus type 1 and type 2 in selected German populations - Relevance for the incidence of genital herpes.
J. Med. Virol., **61** : 201-207 (2000).

200/007-834, 10 - 2025-03