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Deletions: -

LIAISON® VCA IgG (REF 310510)

1. INTENDED PURPOSE

The LIAISON® VCA IgG assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination of specific IgG antibodies to Epstein-Barr virus (EBV) viral capsid antigen (VCA) in human serum and plasma. The assay is intended as an aid in the diagnosis and in the determining the stage of Epstein-Barr Viral infection when performed in conjunction with other EBV markers (EBV IgM, EBNA IgG).
The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

The Epstein-Barr virus (EBV) is a DNA double stranded γ-herpesvirus also known as herpesvirus 4. It is calculated that 95% of the world population is infected with the Epstein-Barr virus, and most individuals are infected by their 30th year of age. A lower prevalence, 20-80%, is observed in preadolescents, with factors such as the geographic area of residence, ethnicity and socioeconomic status considered important in lowering the age of virus acquisition[1-5].

After oral acquisition, EBV infects the oral epithelium, replicates and spreads to contiguous tissues and ultimately to the lymphoreticular system, infecting B cells. After primary infection, the EBV establishes latency in oropharyngeal epithelial cells and in B lymphocytes, where it is maintained in the form of episomal circular DNA, and from where it sometimes reactivates, re-entering a lytic cycle[2,9]. During childhood, primary infection with EBV is often asymptomatic. Acquisition of the virus during adolescence through adulthood results in infectious mononucleosis (IM) in the majority of persons.

Typical symptoms of IM are sore throat, usually severe and accompanied by enlargement of cervical lymph nodes resulting in neck swelling, generalized aching and discomfort, fatigue.

Less frequently, patients can also experience abdominal pain, nausea, vomit, water dropsies in periorbital regions, hepatitis accompanied by hepatomegaly and jaundice[1,3,4,6,10].

Other than EBV, mononucleosis can also be caused by several different pathogens, such as cytomegalovirus, *Toxoplasma gondii*, human herpesvirus 6, adenovirus, human immunodeficiency, rubella, mumps, hepatitis A, B, C and E viruses (mononucleosis caused by agents other than EBV is sometimes called mononucleosis-like illness)[2-5]. Together, the above findings evidence how diagnosis of IM cannot be based solely on clinical observations and highlight the importance of serodiagnosis. Correct EBV diagnosis becomes of especial importance for subjects at risk of complications, since a prompt therapeutic intervention is crucial for effectiveness[3,5].

Seroconversion to anti-EBV specific antigens is recommended for a correct diagnosis of IM caused by EBV infection, as well as for a confirmation of other EBV-caused illnesses. In addition, as the different anti-EBV specific antibodies appear with a known time pattern, their detection in serum or plasma allows an estimate of the timing of infection[4,8-10,13].

Antibodies to EBV follow a distinct pattern of appearance (and, for some of them, decline) following the onset of symptoms, making them helpful for the diagnosis of EBV infections as well as for their stage. IgM antibodies against the viral capsid antigen (VCA) are the first to appear, reaching a peak and usually disappear in two to three months of the onset of disease. IgG anti-VCA antibodies appear during the acute primary infection phase, several weeks or months after the onset of disease and remain life-long after a little decrease between the acute and convalescent phases. IgG antibodies against the EBV nuclear antigen (EBNA) appear weeks after the onset of symptoms and also remain for life. Finally, IgG antibodies against the EBV early antigen-diffuse [EA(D)] appear during the primary infection after anti-VCA IgM antibodies, and disappear after the convalescent phase in ~80% of the infected population[1-4,6,7].

Detection of anti-VCA IgM and anti-VCA IgG, anti-EBNA IgG and anti-[EA-(D)] IgG antibodies is used for diagnosis of EBV primary infections. Considering the time of onset of anti-EBV antibodies, diagnostic interpretation of serological results should be made by personnel with specific training on the basis of antibody combinations, data on low or high antibody titers, and patients' clinical information[1,2,4,8]. Because of the complex relationship that exists between host reaction to EBV and clinical manifestations, tracking of EBV antibody patterns may assist in diagnosis of EBV infection. Antibody response profiles for the different EBV antigens demonstrate a characteristic pattern for silent primary or persistent latent EBV infections, as well as for each of the EBV-associated diseases.

According to some authors, diagnoses might be slightly more complex when unusual antibody kinetics and other factors are taken into consideration, such as the fact that not all primarily infected individuals produce anti-VCA IgM antibodies, or that a minority of individuals do not produce anti-EBNA IgG[3,5,7,14].

A prolonged EBV primary infection, or an EBV reactivation, may become associated with severe complications characterized by an elevated degree of morbidity and mortality. The most at risk categories comprise heavily immunosuppressed individuals and bone marrow/solid organs transplant recipients. EBV seronegative organ receivers are especially at risk when receiving from seropositive donors, as EBV acquisition exposes them to a high risk of developing posttransplant lymphoproliferative disorder[2,3,5,6,11,12].

*(LIAISON®, LIAISON® XL, LIAISON® XS)

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgG to Epstein-Barr viral capsid antigens (VCA) is an indirect chemiluminescence immunoassay (CLIA). p18 synthetic peptide is the major component 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, VCA antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with VCA 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 VCA IgG concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥0.25% solid) coated with Epstein-Barr viral capsid antigens (approx. 100 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (3.2 mL)	CAL 1	Human serum/plasma containing low EBV VCA IgG levels (approx. 37 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (U/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (3.2 mL)	CAL 2	Human serum/plasma containing high EBV VCA IgG levels (approx. 298 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (U/mL) are referenced to an in-house antibody preparation.
Specimen diluent (2 x 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® VCA IgG controls (negative and positive) (REF 310511).

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 labelled as follows:

REAGENTS:	CAL 1, CAL 2, DIL SPE, 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 labelled 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 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

– **Sealed**: stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**: up to eight (8) weeks.
– Use storage rack provided with the Analyzer 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 type of specimen must be used in the assay. The following have been tested and may be used:
– Serum;
– Plasma collected with the following anticoagulant:
  .heparin;
  .potassium EDTA;
  .sodium citrate.

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:
– 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to 6 freeze-thaw cycles, however multiple freeze thaw cycles should be avoided;
– Room temperature sample storage 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 170 µL of 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 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.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL and LIAISON® XS analyzers: 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 specimen diluent and coated magnetic particles.
2. Dispense calibrators, controls or specimens into the reaction module.
3. Incubate.
4. Wash with Wash/System liquid.
5. Dispense conjugate into the reaction module.
6. Incubate.
7. Wash with Wash/System liquid.
8. 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® VCA IgG controls (REF 310511)
(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 VCA IgG antibody concentrations expressed as U/mL 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.

Assay range. 10 to 750 U/mL VCA IgG.
Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:20). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows up to 100 sample predilutions to be performed.

The cut-off value discriminating between the presence and the absence of VCA IgG is 20 U/mL. Sample results should be interpreted as follows:
Samples with VCA IgG concentrations below 20 U/mL should be graded *negative*.
Samples with VCA IgG concentrations equal to or above 20 U/mL should be graded *positive*.

A negative result generally excludes past exposure to Epstein-Barr virus. However, it does not rule out acute infection, since the specimen may have been collected too early during the acute phase, when IgG to VCA levels may still be undetectable. If infection with Epstein-Barr virus is suspected despite a negative finding, a second sample should be collected and tested 10 to 14 days later to look for seroconversion.
A positive result indicates exposure to EBV. In this case, the presence of IgM to EBV and IgG to EBNA should be determined, in order to assess the phase of infection (i.e. acute, convalescent or past infection).

Test results are reported quantitatively as positive or negative for the presence of VCA 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.

Parallel determination of specific VCA IgG, EBNA IgG and EBV IgM levels enables better discrimination between different phases of EBV infection. Whenever multiple LIAISON® EBV tests are performed, a different cut-off may be used for more correct interpretation of EBNA IgG and EBV IgM results. The following interpretation of results is recommended.

EBV IgM result	VCA IgG result	EBNA IgG result	Interpretation
< 20 U/mL	< 20 U/mL	< 20 U/mL	EBV negative.
≥ 20 U/mL	< 20 U/mL	< 20 U/mL	Suspected primary EBV infection (early phase).
≥ 20 U/mL	≥ 20 U/mL	< 20 U/mL	Primary EBV infection (acute phase).
≥ 40 U/mL	≥ 20 U/mL	≥ 20 U/mL	Primary EBV infection (transient phase).
< 40 U/mL	≥ 20 U/mL	≥ 20 U/mL	Past EBV infection or reactivation.
< 20 U/mL	≥ 20 U/mL	≥ 5 U/mL	Past EBV infection or reactivation.
< 20 U/mL	≥ 20 U/mL	< 5 U/mL	Unresolved (VCA IgG positive only).
Other results			Unknown.

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® EBV test is used in conjunction with other manufacturers' assays for detection of specific EBV serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

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, HSV, hHV 6, VZV, parvovirus B19, HAV, *Toxoplasma gondii*, *Mycoplasma pneumoniae* – *(b)* anti-nuclear (ANA) antibodies and 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	E	F	G	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (U/mL)	29.2	36.5	40.8	68.3	78.9	88.3	212	1.71	75.9
Standard deviation	2.43	1.77	1.39	4.88	3.76	3.97	21.16	0.13	3.12
Coefficient of variation (%)	8.3	4.9	3.4	7.1	4.8	4.5	10.0	7.6	4.1
Min. value	25.1	32.3	38.5	58.4	70.8	82.0	174	1.52	71.2
Max. value	34.2	39.2	43.6	77.2	86.3	94.0	238	1.99	81.6

Reproducibility	B	C	D	E	H	I	G	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (U/mL)	35.9	39.4	61.4	70.4	96.9	192	202	2.12	74.2
Standard deviation	3.45	3.74	8.14	9.96	11.68	34.43	42.21	1.02	10.35
Coefficient of variation (%)	9.6	9.5	13.3	14.2	12.0	18.0	20.9	47.8	14.0
Min. value	30.2	33.7	50.2	51.7	77.9	138	89.0	0.893	59.2
Max. value	42.0	46.4	77.4	86.2	119	260	270	4.27	104

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

Reproducibility	LIAISON® VCA IgG (Code 310510) on LIAISON®					
Sample ID	L	M	N	O	P	Positive Control
Mean (U/mL - RLUs)	4150*	28.4	42.9	104	258	75.9
Inter-lot coefficient of variation (%)	26.1	8.4	7.2	15.1	11.4	2.1

* RLUs reported.

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	4	5	6	7	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (U/mL)	26.3	27.7	35.1	40.3	78.8	90.9	220	0.00	80.3
Standard deviation	1.16	0.95	1.17	1.14	3.14	3.96	9.03	0.00	2.67
Coefficient of variation (%)	4.4	3.4	3.3	2.8	4.0	4.4	4.1	–	3.3
Min. value	23.7	25.5	33.0	36.8	73.0	84.0	200	0.00	74.9
Max. value	27.9	29.1	37.2	41.8	84.2	100	234	0.00	85.0

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

Reproducibility	2	3	4	5	8	9	7	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (U/mL)	33.4	41.6	47.2	98.0	125	225	276	0.00	95.9
Standard deviation	3.59	2.97	4.60	14.30	11.84	21.85	30.64	0.00	9.51
Coefficient of variation (%)	10.7	7.1	9.8	14.6	9.5	9.7	11.1	–	9.9
Min. value	27.4	37.4	40.7	77.7	102	191	224	0.00	79.8
Max. value	40.2	47.0	55.9	134	140	262	325	0.00	120

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

Reproducibility	LIAISON® VCA IgG (Code 310510) on LIAISON® XL					
Sample ID	17	18	19	20	21	Positive Control
Mean (U/mL - RLUs)	9714*	29.0	40.8	116	271	80.6
Inter-lot coefficient of variation (%)	16.3	10.3	9.9	9.9	11.9	3.6

* RLUs reported.

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

A coded panel comprised of seven (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 LIAISON® Control VCA 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 value (U/mL), 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. 7 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	10	11	12	13	14	15	16	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (U/mL)	13.8	28.7	32.0	40.4	97.8	279	403	7304	83.9
Standard deviation	0.32	0.40	0.39	0.70	2.32	10.7	14.7	224	1.83
Coefficient of variation (%)	2.3	1.4	1.2	1.7	2.4	3.8	3.7	3.1	2.2
Min. value (U/mL)	12.1	26.0	29.9	36.9	86.3	194	328	6171	67.0
Max. value (U/mL)	15.9	31.1	35.0	44.3	107	302	439	8695	97.4

*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. 7 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	10	11	12	13	14	15	16	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (U/mL)	13.8	28.7	32.0	40.4	97.8	279	403	7304	83.9
Standard deviation	0.64	0.95	0.94	1.40	3.83	12.46	16.9	567	5.86
Coefficient of variation (%)	4.6	3.3	2.9	3.5	3.9	4.5	4.2	7.8	7.0
Min. value (U/mL)	12.1	26.0	29.9	36.9	86.3	194	328	6171	67.0
Max. value (U/mL)	15.9	31.1	35.0	44.3	107	302	439	8695	97.4

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

15.5. Linearity and Trueness

The assay linearity has been checked by the dilution test.

Dilution test. Four serum samples containing high VCA IgG concentrations were tested as such and after serial dilution with the specimen diluent. VCA IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.991 to 0.999.

Dilution	Expected concentration, U/mL	Measured concentration, U/mL	% Recovery	Dilution	Expected concentration, U/mL	Measured concentration, U/mL	% Recovery
neat	–	311.0	–	neat	–	662.0	–
1:2	155.5	150.0	96.5	1:2	331.0	313.0	94.6
1:4	77.8	78.0	100.3	1:4	165.5	140.0	84.6
1:8	38.9	36.0	92.5	1:8	82.8	78.0	94.2
1:16	19.4	16.0	82.5	1:16	41.4	34.0	82.1
1:32	9.7	8.0	82.5	1:32	20.7	13.0	62.8
neat	–	669.0	–	neat	–	894.0	–
1:2	334.5	251.0	75.0	1:2	447.0	521.0	116.5
1:4	167.3	118.0	70.5	1:4	223.5	193.0	86.5
1:8	83.6	61.0	73.0	1:8	111.8	111.0	99.3
1:16	41.8	33.0	78.9	1:16	55.9	54.0	96.6
1:32	20.9	19.0	90.9	1:32	27.9	26.0	93.2

The assay trueness has been checked by recovery test.

One set formed of a high- and a low- concentration VCA IgG sample (samples X and Y) was mixed in 1:2, 1:1 and 2:1 ratios and assayed. Percent recoveries were determined from results of undiluted samples. Measured versus expected VCA IgG concentrations were analyzed by linear regression. The observed correlation coefficients (r) was 0.996.

Set 1	Expected concentration, U/mL	Measured concentration, U/mL	% Recovery
X neat	-	15.80	-
2:1	196.0	230.0	117.3
1:1	288.9	324.0	112.1
1:2	380.1	408.0	107.3
Y neat	-	562.0	-

15.6. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve). Analysis of saturation effect was evaluated by testing three high-titred samples positive for VCA IgG. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.7. Limit of Blank (LoB)*

Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® VCA IgG assay is 1.44 U/mL.

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

15.8. Limit of Detection (LoD)

Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® VCA IgG assay is 2.24 U/mL.

15.9. Limit of Quantification (LoQ)

Following the method from CLSI EP17-A2, the limit of quantification for the LIAISON® VCA IgG assay is 3.11 U/mL (defined as the lowest analyte concentration that can be determined with an inter-assay CV <20%).

15.10. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 2149 specimens from different selected populations (subjects never infected by EBV, apparently healthy subjects, subjects affected by autoimmune diseases, patients affected by other infectious diseases with similar symptomatology, patients affected by primary EBV infection, subjects with past EBV infection, patients with suspected chronic EBV infection, patients affected by reactivated EBV infection). 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. 55 specimens were unresolved either by the method under test or by the reference methods and therefore were not included in the data analysis.

Ten positive and 227 negative results were observed in the expected negative population studied - diagnostic specificity: 95.78% (95% confidence interval: 92.38-97.96%).

28 negative and 1829 positive results were observed in the expected positive population studied - diagnostic sensitivity: 98.49% (95% confidence interval: 97.83-99.00%).

15.11. EBV reactivity pattern

2343 samples from different selected populations were tested during performance evaluation studies: subjects never infected by EBV, apparently healthy adult subjects, subjects affected by autoimmune diseases, patients affected by other infectious diseases, patients affected by primary EBV infection, subjects with past EBV infection, patients with suspected chronic EBV infection, patients affected by reactivated EBV infection.

The three LIAISON® EBV test results were combined to identify the phase of EBV infection from a serological point of view and to evaluate the ability of multiple LIAISON® EBV tests to correctly classify the samples.

The serological diagnosis arising from multiple LIAISON® EBV tests was compared with the results obtained from the comparison tests for the most representative categories of subjects, such as EBV-negative subjects, patients affected by primary EBV infection and subjects with past EBV infection.

Out of 210 expected EBV-negative samples, 181 samples showed a negative LIAISON® EBV pattern. Concordance with the comparison pattern was therefore 86.20% (95% confidence interval: 80.77%-90.56%).

Out of 282 expected primary EBV infection samples, 255 samples showed a pattern of primary EBV infection by LIAISON®. Concordance with the comparison pattern was therefore 90.43% (95% confidence interval: 86.37%-93.59%).

Out of 1616 expected past EBV infection samples, 1479 samples showed a pattern of past EBV infection by LIAISON®. Concordance with the comparison pattern was therefore 91.52% (95% confidence interval: 90.05%-92.84%).

Interpretation of results obtained with multiple LIAISON® EBV tests can be directly performed by the instrument through Dia-Link software.

LIAISON® EBV pattern	EBV IgM	VCA IgG	EBNA IgG	No. of subjects	Percentage
EBV-negative subjects	negative	negative	negative	202	8.6%
Primary EBV infection					
. early phase	positive	negative	negative	113	4.8%
. acute phase	positive	positive	negative	221	9.4%
. transient phase	positive	positive	positive	132	5.6%
Past EBV infection	negative	positive	positive	1594	68.1%
Unresolved EBV pattern	negative	positive	negative	32	1.4%
Unknown EBV pattern	negative	negative	positive	49	2.1%
TOTAL				2343	100%

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.

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