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Changes: §1, §2, §4, §5, §6, §7, §8, §9, §10, §12, §15.3, §15.4, References;
Deletions: §14;

LIAISON® CMV IgG Avidity II (REF 310765)

1. INTENDED PURPOSE

The LIAISON® CMV IgG Avidity II assay uses indirect chemiluminescent immunoassay (CLIA) technology for the in vitro determination of antigen-binding avidity of IgG antibodies to human Cytomegalovirus in human serum and plasma samples. The assay is intended as an aid in the diagnosis and staging of hCMV infection, in subjects selected positive for the presence of hCMV IgG and hCMV IgM. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

CMV is a host-restricted member of the Herpesviridae family of viruses. Primary infection is characterized by a period of active virus replication with virus shedding in saliva, urine, milk, and genital secretions, a viremic phase. CMV infection is endemic and without seasonal variation[1].

In immunocompetent hosts, CMV infections are generally subclinical. However, when infection occurs during pregnancy without consequences for the mother, it can have serious repercussions for the fetus. In immunocompetent mothers, reactivation of endogenous virus and/or reinfection with new strains occurs periodically, and DNAemia and viruria may be present in both[2]. Less than 5% of pregnant women with proven primary CMV infections are symptomatic, with an even smaller percentage manifesting mononucleosis-like syndrome. Clinical manifestations have not been reported with recurrent infections (reactivations or reinfections). Serologic assays IgG and IgM are the primary tools for assessing primary CMV infections during pregnancy.

The diagnosis of primary CMV infection can be easily confirmed by documenting seroconversion (i.e., the de novo appearance of virus-specific IgG antibodies in a pregnant woman who was seronegative). In the absence of serologic screening, this is seldom available in clinical practice. The presence of IgG antibodies denotes past infection from 2 weeks to years in duration. CMV IgM antibodies are present during primary and non-primary infections, and thus, are not really informative for determining seroconversion.

The IgM antibody response varies widely from one patient to another. IgM Seropositivity can be detected up to 16 weeks, but it is unusual to last more than 1 year. It is typical to see sharp drops in titers within the first 2 to 3 months of infection. More sensitive assays of IgM antibodies have detected maternal CMV-specific IgM antibodies up to 1 year from enrollment in clinical studies.

The CMV IgG avidity assay is considered a primary tool to date the timing of an infection. This test is based on the notion that IgG avidity increases with time; low-avidity IgGs are associated with recent infections, while a high avidity index indicates past infections. This assay is based on the observation that IgG antibodies of low avidity are present during the first months after the onset of infection.

In determining the risk of congenital CMV, a moderate-to-high avidity index obtained before the 18th week of gestation has a negative predictive value of 100%. When the avidity index is determined between 21 and 23 weeks of gestation, the negative predictive value dropped to 91%. The explanation for this observation is that some of the women who transmitted the infection in utero had acquired the infection at a very early gestational age. One important limitation of early studies using the IgG avidity test was the lack of standardization. In one study, the ability of these IgG avidity assays to identify primary CMV infection almost reached 100%, whereas the ability to exclude a recent infection ranged from 20% to 96%. When coupled with the detection of CMV specific IgM antibodies, the avidity test has been used to estimate risk of primary infection and damaging congenital infection. This approach has been extensively used in Europe and now represents a component of routine testing in pregnant women, although there remains concern about the standardization of the various assays. However, it is not widely used in the United States, presumably secondary to the lack of widespread screening for CMV infections in pregnant women.

*(LIAISON®, LIAISON® XL, LIAISON® XS).

3. PRINCIPLE OF THE PROCEDURE

The method for determination of antigen-binding avidity of specific IgG to hCMV is an indirect chemiluminescence immunoassay (CLIA). hCMV antigen is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). The presence of strong bonds between IgG antibodies and hCMV (i.e., IgG avidity) in a given IgG-positive sample is detected by comparing the signal of a reference (i.e., non-treated) sample with the signal of the same sample after treating with urea, which dissociates weak bonds between IgG and hCMV. During the first incubation, hCMV antibodies present in calibrators, samples or controls bind to the solid phase (reference and treated samples). During the second incubation, the dissociating agent changes antigen-antibody bonds (treated sample only). Only high-avidity antibodies remain bound to the solid phase, whereas low-avidity antibodies are eliminated. During the final incubation, the antibody conjugate reacts with hCMV IgG already bound to the solid phase (reference and treated samples). 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 hCMV IgG concentration present in calibrators, samples or controls. The IgG avidity index is given by the ratio of urea-treated specimens to reference specimens.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (1.5 mL)	SORB	Magnetic particles (≥0.25% solid) coated with inactivated hCMV antigen (AD 169 strain) (approx. 0.25 mg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.3 mL)	CAL 1	Human serum/plasma containing low hCMV IgG levels (approx. 130 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentration is referenced to an in house antigen preparation.
Calibrator 2 (2.3 mL)	CAL 2	Human serum/plasma containing high hCMV IgG levels (approx. 3000 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentration is referenced to an in house antigen preparation.
Specimen diluent (28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye.
Buffer B (13 mL)	BUF B	TRIS buffer, urea, 0.2% ProClin™ 300, preservatives, an inert blue 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		25

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® Control CMV IgG Avidity II (low- and high-avidity) (REF 310766).

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.

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

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

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

REAGENTS:	CAL 1, CAL 2, DIL SPE, BUF B, 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.

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 Analyzer and LIAISON® XS analyzers

– LIAISON® XL 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 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;
– plasma collected with the following anticoagulants:
  .lithium heparin;
  .sodium heparin;
  .K2-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:
– 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 6 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 170 µL of specimen (20 µL specimen + 150 µL dead volume).

Only specimens positive for hCMV IgG (above 14.0 U/mL with LIAISON® CMV IgG II REF 310745 assay) should be tested for IgG avidity: lower-concentration samples may give rise to sample misclassification.

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 (8) weeks before.
– Control values lie outside the expected ranges.
– **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 local DiaSorin technical support or representative.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL Analyzer: Calibrator values are stored in the 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

This test requires the following assay files: CGAvII, CNTII and C-TII.

To test specimens use CGAvII.

Never use CNTII or C-TII.

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.

Specimens must be tested with both protocols in order to obtain the final results (avidity index). The analyzer operations are as follows:

Protocol A

1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense coated magnetic particles.
3. Dispense specimen diluent.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Kit and measure the light emitted.

Protocol B

1. Dispense controls or specimens into the reaction module (calibration is not performed).
2. Dispense coated magnetic particles.
3. Dispense specimen diluent.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense buffer B.
7. Incubate.
8. Wash with Wash/System liquid.
9. Dispense conjugate into the reaction module.
10. Incubate.
11. Wash with Wash/System liquid.
12. 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® CMV IgG Avidity II controls (REF 310766)
(a) at least once per day of use,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
or in agreement with guidelines or requirements of local regulations or accredited organizations.

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

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

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates the antigen-binding avidity index for specific IgG to hCMV (ratio of urea-treated specimens to reference specimens) 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. 0.000 to 0.950 avidity index (Av) of hCMV IgG.
Samples containing hCMV IgG antibody levels above the assay range (higher than 140 U/mL) by LIAISON® CMV IgG II (REF 310745) should be prediluted with specimen diluent by the Dilute function of the instrument before performing CMV IgG Avidity test. The recommended dilution factor is 1:10. When the 1:10 diluted samples still score above the assay range of the avidity test, CMV IgG Avidity test should be repeated after prediluting the samples 1:20.
Samples containing hCMV IgG antibody levels within the assay range may be tested directly. In case the samples show levels above the assay range of CMV IgG Avidity test (i.e., alert Invalid Combi Partner displayed), they should be prediluted 1:10 and tested again.
The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens and the avidity index will be calculated. The specimen diluent excess available in the reagent integral allows up to 25 sample predilutions to be performed.

Sample results should be interpreted as follows:
An avidity index value for hCMV IgG below 0.150 should be graded as *low avidity*.
An avidity index value for hCMV IgG ranging between 0.150 and 0.250 should be graded *moderate avidity*.
An avidity index value for hCMV IgG equal to or above 0.250 should be graded as *high avidity*.

Samples with avidity index greater than 0.950 should be retested. If the avidity index value is confirmed, it should be graded as *high avidity*.

Samples giving an Invalid Combi Partner Alert (for LIAISON® Analyzer platform) or a Failed avidity (for LIAISON® XL and LIAISON® XS platforms) with a result of C-TII << 0.00 U/mL (< 0 U/mL for LIAISON® XL and LIAISON® XS Analyzers) for urea-treated specimen should be retested. If, after the retest, this result is confirmed, sample should be graded as *low avidity*.

A low avidity index value suggests the possibility of primary infection acquired less than three months before sample collection. A low avidity index, however, does not exclude past infection, as a proportion of infected persons may exhibit persistence of low-avidity IgG antibodies for months.
A moderate avidity index value does not rule out the possibility of recent infection but may indicate past infection with unaccomplished maturity of IgG avidity.
A high avidity index value may exclude that primary infection was acquired less than three months before sample collection. Serological data from detection of additional hCMV markers may provide useful information for clinical interpretation of results.

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

- Repeat testing of samples with avidity index values close to the discrimination threshold (i.e., 0.150 and 0.250 Av) may show some fluctuations in specimen classification, depending on precision of the system. This expected behaviour does not affect performance characteristics of the assay.
- 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).

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, Sodium and Lithium 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.

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	C	D	E	F	G	H	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.070	0.093	0.116	0.122	0.292	0.623	0.687	0.711	0.048	0.713
Standard deviation (Av)	0.004	0.007	0.011	0.009	0.021	0.044	0.038	0.026	0.007	0.053
Coefficient of variation (%)	5.4	7.4	9.4	7.5	7.1	7.0	5.5	3.7	15.7	7.4
Min. value (Av)	0.065	0.081	0.099	0.105	0.268	0.569	0.630	0.661	0.039	0.567
Max. value (Av)	0.081	0.105	0.140	0.141	0.366	0.734	0.762	0.760	0.064	0.782

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

Reproducibility - Site 1	A	B	C	D	E	F	G	H	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.084	0.102	0.122	0.136	0.293	0.635	0.714	0.725	0.043	0.726
Standard deviation (Av)	0.010	0.009	0.008	0.009	0.014	0.043	0.050	0.039	0.006	0.048
Coefficient of variation (%)	11.4	8.7	6.4	6.6	4.8	6.8	7.0	5.4	14.5	6.7
Min. value (Av)	0.071	0.089	0.111	0.118	0.264	0.584	0.635	0.672	0.029	0.657
Max. value (Av)	0.103	0.121	0.144	0.153	0.318	0.746	0.833	0.846	0.055	0.813

Reproducibility - Site 2	A	B	C	D	E	F	G	H	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.073	0.089	0.112	0.122	0.289	0.634	0.704	0.728	0.039	0.758
Standard deviation (Av)	0.003	0.002	0.005	0.004	0.007	0.019	0.019	0.018	0.005	0.029
Coefficient of variation (%)	4.0	2.4	4.4	3.3	2.5	3.0	2.7	2.5	14.0	3.9
Min. value (Av)	0.069	0.085	0.105	0.116	0.270	0.591	0.660	0.692	0.034	0.714
Max. value (Av)	0.080	0.094	0.121	0.131	0.302	0.663	0.739	0.779	0.059	0.840

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	8	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.084	0.102	0.124	0.131	0.285	0.624	0.650	0.743	0.051	0.784
Standard deviation (Av)	0.003	0.003	0.004	0.005	0.011	0.018	0.027	0.034	0.002	0.024
Coefficient of variation (%)	3.8	3.2	3.4	3.5	3.7	2.9	4.1	4.5	4.1	3.0
Min. value (Av)	0.077	0.096	0.118	0.123	0.268	0.597	0.595	0.681	0.048	0.743
Max. value (Av)	0.089	0.108	0.131	0.140	0.309	0.654	0.699	0.814	0.055	0.831

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

Reproducibility - Site 1	1	2	3	4	5	6	7	8	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.081	0.104	0.126	0.140	0.283	0.631	0.705	0.758	0.049	0.764
Standard deviation (Av)	0.004	0.006	0.006	0.006	0.009	0.027	0.022	0.041	0.002	0.035
Coefficient of variation (%)	4.5	6.1	4.6	4.6	3.3	4.2	3.2	5.4	4.7	4.6
Min. value (Av)	0.074	0.089	0.117	0.130	0.265	0.593	0.662	0.688	0.044	0.709
Max. value (Av)	0.088	0.111	0.142	0.150	0.301	0.676	0.740	0.838	0.052	0.837

Reproducibility - Site 2	1	2	3	4	5	6	7	8	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20	20
Mean (Av)	0.078	0.101	0.117	0.127	0.277	0.614	0.704	0.727	0.047	0.750
Standard deviation (Av)	0.006	0.007	0.009	0.009	0.016	0.046	0.045	0.051	0.006	0.072
Coefficient of variation (%)	8.1	7.2	7.3	7.2	5.6	7.5	6.4	7.1	12.7	9.7
Min. value (Av)	0.062	0.078	0.104	0.107	0.236	0.513	0.586	0.625	0.033	0.572
Max. value (Av)	0.091	0.111	0.132	0.146	0.296	0.701	0.779	0.827	0.059	0.920

Lot-to-Lot Reproducibility. Samples tested in singleton on five different LIAISON® XL instruments on at least four different batches.

Reproducibility	LIAISON® CMV IgG Avidity II (Code 310765) on LIAISON® XL					
Sample ID	A	B	C	D	Control Low	Control High
Mean (Av)	0.568	0.595	0.469	0.501	0.073	0.719
Inter-lot coefficient of variation (%)	6.3	4.0	0.0	11.9	19.8	5.8

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® CMV IgG Avidity II Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.
A coded panel comprised of 7 frozen samples containing different concentration of analyte and kit controls was used for the study.
The LIAISON® Control CMV IgG Avidity II 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 dose mean, 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 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	9	10	11	12	13	14	15	Control Low	Control High
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (Av)	0.0458	0.0753	0.177	0.151	0.191	0.488	0.808	0.0556	0.789
Standard deviation	0.002	0.002	0.005	0.004	0.007	0.019	0.027	0.002	0.017
Coefficient of variation (%)	3.5	3.3	3.0	2.8	3.4	3.9	3.4	3.5	2.2
Min. value (Av)	0.0400	0.0692	0.159	0.134	0.168	0.740	0.712	0.0489	0.698
Max. value (Av)	0.0512	0.0822	0.197	0.165	0.223	0.554	0.870	0.0629	0.869

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 6 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	9	10	11	12	13	14	15	Control Low	Control High
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (Av)	0.0458	0.0753	0.177	0.151	0.191	0.488	0.808	0.0556	0.789
Standard deviation	0.002	0.003	0.006	0.005	0.009	0.020	0.030	0.003	0.024
Coefficient of variation (%)	4.1	3.7	3.6	3.6	4.5	4.1	3.7	4.6	3.0
Min. value (Av)	0.0400	0.0692	0.159	0.134	0.168	0.740	0.712	0.0489	0.698
Max. value (Av)	0.0512	0.0822	0.197	0.165	0.223	0.554	0.870	0.0629	0.869

15.5. Trueness

The assay trueness has been checked by the dilution test.

Dilution test. Four high avidity serum samples and four low avidity serum samples containing hCMV IgG were tested as such and after serially diluting with the specimen diluent. Avidity index values for hCMV IgG measured before and after specimen dilution did not result in discrepant sample classification.

Avidity	Dilution	Measured avidity index	Avidity	Dilution	Measured avidity index
Low	neat	0.131	High	neat	0.572
	1:2	0.140		1:2	0.541
	1:5	0.118		1:5	0.620
	1:10	0.072		1:10	0.639
	1:20	-		1:20	0.605
Low	neat	0.120	High	neat	0.716
	1:2	0.143		1:2	0.889
	1:5	0.136		1:5	0.879
	1:10	0.089		1:10	0.799
	1:20	-		1:20	0.853
Low	neat	0.062	High	neat	0.548
	1:2	0.075		1:2	0.592
	1:5	0.067		1:5	0.633
	1:10	0.045		1:10	0.610
	1:20	0.037		1:20	0.680
Low	neat	0.039	High	neat	0.517
	1:2	0.031		1:2	0.612
	1:5	0.032		1:5	0.755
	1:10	0.047		1:10	0.655
	1:20	0.036		1:20	0.680

15.6. Diagnostic concordance

182 specimens were tested from subjects that underwent CMV Avidity testing in different european laboratories.

Estimated time of infection was established taking into account the results of a CE mark reference assay (LIAISON® CMV IgG Avidity REF 310760).

22 specimens were unresolved by the reference method and therefore were not included in the data analysis.

39 low avidity results were observed in the population studied, who presumably acquired the infection in the three months prior to sample collection - diagnostic concordance was 100% (95% confidence interval: 90.98-100%).

1 low avidity, 3 moderate avidity and 117 high avidity results were observed in the population studied, who presumably acquired the infection more than 3 months prior to sample collection - diagnostic concordance was 96,58% (95% confidence interval: 91.75-99.09%).

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.

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