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Deletions: §14;

LIAISON® CMV IgM II (REF 310755)

1. INTENDED PURPOSE

The LIAISON® CMV IgM II assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro semi-quantitative determination of specific IgM antibodies to hCMV in human serum and plasma samples included specimens collected post-mortem (non-heart beating). The assay is intended as an aid in diagnosis of hCMV infection in patients affected by primary hCMV infection, patients with past hCMV infection, patients with long-lasting hCMV IgM including pregnant women, and patients affected by other infectious diseases with similar symptoms. The test can also be used as a screening test for blood and hemocomponents donors as well as for organ, tissue and cells post-mortem donors.
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 enrolment 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.

3. PRINCIPLE OF THE PROCEDURE

The method for semi-quantitative determination of specific IgM to hCMV is an indirect chemiluminescence immunoassay (CLIA). hCMV 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, hCMV antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with hCMV 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 hCMV 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 hCMV or from rheumatoid factor.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with inactivated hCMV antigen (AD 169 strain) (approx. 0.4 mg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (0.6 mL)	CAL 1	Human serum/plasma containing low hCMV IgM levels (approx. 20 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 (0.6 mL)	CAL 2	Human serum/plasma containing high hCMV IgM levels (approx. 60 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.
Buffer A (25 mL)	BUF A	Goat IgG to human IgG (absorbent reagent) (≥ 5%), 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 (minimum 10 ng/mL), 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® CMV IgM II controls (negative and positive) (REF 310756).

5. WARNINGS AND PRECAUTIONS

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

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

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

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

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

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

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

REAGENTS:	CAL 1, CAL 2, BUF 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. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

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

An incomplete magnetic particles resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:
Visually inspect the reagents, calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

– Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS analyzers

– LIAISON® XL and LIAISON® XS analyzers are equipped with 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.

CONTROLS

Refer to the LIAISON® CMV IgM II Control Set instructions for use section for proper preparation and handling instructions.

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**: Minimum stability eight (8) weeks.
– Use storage rack provided with the LIAISON® Analyzer family for upright storage of reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate later proper resuspension of magnetic particles.

Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

The correct type of specimen must be used with the assay. The following have been tested and may be used:
– Serum,
– Plasma collected with the following anticoagulant:
  .Lithium heparin;
  .Sodium heparin;
  .K2-EDTA;
  .Sodium citrate.

Post-mortem specimens, collected up to 24 hours after death, have been tested and may be also used in the assay. Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation.

Centrifugation conditions range from 1,000 to 3,000 g for 10 minutes. Conditions may vary depending on tube manufacturers recommendations. Use of alternate 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.

Before shipping specimens, serum or plasma specimens should be removed from clot, red cells or gel separator. Specimens may be shipped in dry ice (frozen), in wet ice (for 2°-8°C) or at room temperature (20°-25°C), by following 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:
– up to 48h at room temperature, however it should be evaluated and validated by the laboratory;
– 2°-8°C for seven (7) days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– up to six (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 stored at room temperature (20°-25°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.

Cadaveric specimens should be stored in the following storage conditions:
– room temperature must be avoided;
– 2°-8°C for seven (7) days; otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to six (6) freeze-thaw cycles, however multiple freeze thaw cycles should be avoided.

The minimum volume required 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 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 DiaSorin Technical support or representative.

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

11. ASSAY PROCEDURE

For LIAISON® XL Analyzer only: this test requires the following assay files: CMVMII, CMV-MII and CMVMII22.
To test specimens use CMV-MII or CMVMII22.
Never use CMVMII.

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 and controls with buffer A.
2. Dispense coated magnetic particles and Buffer A into the reaction module.
3. Dispense calibrators, controls or specimens into the reaction module.
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

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® CMV IgM II controls (REF 310756)
(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 beyond eight weeks, 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 hCMV IgM 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. 5.0 to 140 U/mL hCMV IgM.
Sample results should be interpreted as follows:
Samples with hCMV IgM concentrations below 18.0 U/mL should be graded *negative*.
Samples with hCMV IgM concentrations ranging between 18.0 and 22.0 U/mL should be graded *equivocal*.
Samples with hCMV IgM concentrations equal to or above 22.0 U/mL should be graded *positive*.

Assay file for hCMV IgM determination on LIAISON® XL Analyzer with the above interpretation of results is **CMV-MII.**

A positive result is indicative of the presence of IgM from either primary, or recurrent, or past infection with persistent IgM levels.
An equivocal result may be indicative of the presence of low hCMV IgM levels. Serological data from detection of additional hCMV markers (e.g., IgG avidity) may provide useful information for clinical interpretation of results.
A negative result is indicative of the absence of detectable IgM, but does not always rule out acute hCMV infection. If clinical exposure to hCMV is suspected despite a negative finding, a second sample should be collected and tested no less than one or two weeks later.

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.

Note - The LIAISON® CMV IgM II kit is designed to measure the concentration of hCMV IgM with highest sensitivity, thus allowing very early detection of antibodies at the onset of acute infection. As low hCMV IgM levels in recurrent infection may persist over time and samples containing low levels of long-lasting IgM score equivocal or positive in the test, laboratories should adopt additional diagnostic procedures, like IgG avidity determination and/or different IgM assays, as useful tools to interpret IgM reactivity.
Laboratories may also find it useful not to apply the equivocal zone to increase diagnostic specificity of the LIAISON® CMV IgM II kit. High to medium levels of hCMV IgM antibodies generally appear in the early stage of infection, while long-lasting IgM typically persist at lower levels before disappearing: the window of IgM positivity during the course of infection is then stretched towards the early phase. The risk of undetected acute infection samples remains negligible and further minimized by the practice of collecting a second sample one or two weeks later from negative patients.
In case this option is chosen, sample results should be interpreted as follows:
Samples with hCMV IgM concentrations below 22.0 U/mL should be graded *negative*.
Samples with hCMV IgM concentrations equal to or above 22.0 U/mL should be graded *positive*.

For LIAISON® Analyzer, to adopt the option at 22.0 U/mL please contact local DiaSorin representatives.

For LIAISON® XL Analyzer, to adopt the option at 22.0 U/mL please use assay file **CMVMII22.**

Refer to the table in section 15.6 for details on results observed during performance evaluation.

14. LIMITATIONS OF THE PROCEDURE

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Bacterial contamination or heat inactivation of the specimens may affect the test results.

Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS,). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Before testing cadaveric specimens, collection and centrifugation procedures should be carefully applied. After death, hemolysis and other changes (including proteolysis and dilution) occur in blood, which may lead to False Negative and False Positive in testing. In subjects transfused immediately prior to death high percentage of haemodilution can affect the performance of the test due to analyte dilution.

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, 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), Total protein (High) (up to 120 g/L), Total protein (Low) (up to 60 g/L), Total IgG (up to 2000 mg/dL), Total IgM (up to 200 mg/dL), or by freeze-thaw cycles of samples. In addition, controlled studies of potentially exogenous substances showed no interference to each substance listed below in the LIAISON® CMV IgM II up to the indicated concentration.

Tested Compound	Tested concentration
Vitamin A	800 µg/dL
Vitamin B12	2850 pg/mL
Vitamin C	20 mg/dL
Vitamin D	450 ng/mL
Vitamin E	120 mg/L
Vitamin H (Biotin)	3510 ng/mL
Folic Acid	160 ng/mL
Acetaminophen	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Acetylsalicylic acid	3 mg/dL
Caffeine	10.8 mg/dL
Ethanol	600 mg/dL
Prednisolone	0.12 mg/dL
Hydrocortisone	200 mg/L
Amiodarone	4.20 mg/dL
Atropine	0.06 mg/dL
Dopamine	0.0621 mg/dL
Epinephrine	0.25 mg/dL
Norepinephrine	50.7 ng/dL

Cross-reactions. The cross-reactivity study for the LIAISON® CMV IgM II assay was designed to evaluate potential interference from antibodies to other organism that may cause infectious diseases (EBV, Rubella, parvovirus B19, Toxoplasma gondii, HBV, HSV, HAV, Treponema pallidum, VZV, measles and mumps virus, Borrelia burgdorferi), β-herpes (HHV6/HHV7), human IgG anti-CMV (high titer) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies (ANA), rheumatoid factor antibodies (RFantibodies). Samples for these studies were pre-screened with another commercially available hCMV IgM assay. If found negative for hCMV 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.

Clinical condition	Number of expected negative samples	LIAISON® equivocal results	LIAISON® positive results
EBV IgM antibodies	126	3	2
Rubella virus IgM antibodies	20	1	1
Parvovirus B19 IgM antibodies	108	7	1
HAV IgM antibodies	50	0	0
Toxoplasma gondii IgM antibodies	9	0	0
HSV-1/2 IgM antibodies	14	0	0
VZV IgM antibodies	20	1	0
Measles virus IgM antibodies	9	0	0
Mumps virus IgM antibodies	20	0	0
Borrelia IgM antibodies	34	0	0
HBc IgM	27	0	0
Syphilis IgM antibodies	9	0	1
Anti-nuclear autoantibodies (ANA)	36	0	0
HAMA antibodies	15	0	0
RF antibodies	32	0	1
β-herpes (HHV6/HHV7)	9	0	0
Human IgG anti-CMV (high titer)	10	0	0
Total	548	12	6

The specificity observed in the selected cross reaction population is not significantly different to what observed in a prospective population. However some of the tested conditions returned results that may be consistent with a conclusion of cross reactivity since the specific reactive samples frequency was higher than the global one. Assay interference may occur due to presence of IgM from patient with early acute infection. Long-lasting IgM should not interfere in LIAISON® CMV IgM II assay.

The reactivity could be related to cross reactions with early IgM of other viruses or to the reapperance of CMV IgM due to polyclonal activation induced by other agents that produce a CMV infection-like diseases.

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. **The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.**

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

Repeatability	1	2	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (U/mL)	< 5.0	6.5	18.7	23.3	25.2	23.8	25.2	48.2	50.1	< 5.0	28.5
Standard deviation (U/mL)	0.15	0.81	0.55	0.58	0.69	0.77	0.68	1.91	1.12	0.00	0.89
Coefficient of variation (%)	N.A.	12.5	2.9	2.5	2.7	3.2	2.7	4.0	2.2	N.A.	3.1
Min. value (U/mL)	1.8	5.6	17.2	22.3	24.1	21.7	23.8	45.1	47.9	0.0	27.1
Max. value (U/mL)	2.4	9.1	19.4	24.4	26.2	24.6	26.6	52.2	51.8	0.0	30.0

Reproducibility. Twenty replicates were performed in different days (maximum of two runs per day) on one integral lot to evaluate reproducibility. The tests were performed in two sites, in house (site 1) and in an independent laboratory (site 2) using two different instruments.

Reproducibility - Site 1	1	2	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (U/mL)	< 5.0	8.0	20.7	25.4	27.8	25.5	28.3	54.8	58.5	< 5.0	29.9
Standard deviation (U/mL)	0.21	1.78	1.30	0.50	0.82	0.84	1.03	2.25	2.18	0.00	0.83
Coefficient of variation (%)	N.A.	22.1	6.3	2.0	2.9	3.3	3.6	4.1	3.7	N.A.	2.8
Min. value (U/mL)	1.5	5.5	19.4	24.2	26.1	23.9	26.9	50.9	53.7	0.00	28.5
Max. value (U/mL)	2.3	11.8	25.4	26.4	29.3	27.7	30.2	58.3	60.9	0.00	31.3

Reproducibility - Site 2	1	2	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (U/mL)	< 5.0	7.5	19.4	24.0	25.1	25.5	28.8	52.0	55.2	< 5.0	28.2
Standard deviation (U/mL)	0.42	1.13	0.54	0.96	0.82	0.95	1.29	2.23	1.63	0.00	0.99
Coefficient of variation (%)	N.A.	15.1	2.8	4.0	3.3	3.7	4.5	4.3	3.0	N.A.	3.5
Min. value (U/mL)	1.5	5.6	17.8	21.9	23.9	23.7	24.8	49.1	52.1	0.0	26.4
Max. value (U/mL)	3.2	10.8	20.2	25.6	26.8	27.3	30.5	56.8	58.2	0.0	30.0

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

Reproducibility	LIAISON® CMV IgM II (Code 310755) on LIAISON®							
Sample ID	Negative Control*	Positive Control	10*	11	12	13	14	15
Mean (U/mL)	696	38.5	1351	15.2	18.0	30.1	40.2	41.7
Inter-lot coefficient of variation (%)	5.9	0.6	9.6	5.3	4.1	3.2	7.4	2.9

* Negative control and 10 are expressed in RLU because their doses score out of the assay range.

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. **The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.**

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

Repeatability	1	2	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (U/mL)	< 5.0	8.6	20.2	25.5	24.4	31.2	35.0	51.4	53.2	< 5.0	31.1
Standard deviation (U/mL)	0.17	0.42	0.39	0.60	0.49	0.96	1.63	0.79	0.96	0.08	0.62
Coefficient of variation (%)	N.A.	4.9	2.0	2.3	2.0	3.1	4.7	1.5	1.8	N.A.	2.0
Min. value (U/mL)	1.8	8.2	19.5	24.4	23.3	29.3	33.0	50.0	51.0	0.0	29.9
Max. value (U/mL)	2.4	10.0	21.2	26.8	24.9	33.3	39.8	53.3	55.0	0.3	32.2

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

Reproducibility	1	2	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20	20	20
Mean (U/mL)	< 5.0	9.7	21.7	28.7	27.0	34.7	40.2	54.9	59.0	< 5.0	35.6
Standard deviation (U/mL)	0.42	0.77	0.72	2.01	0.90	2.51	2.20	2.30	2.63	0.32	2.15
Coefficient of variation (%)	N.A.	7.9	3.3	7.0	3.3	7.2	5.5	4.2	4.5	N.A.	6.0
Min. value (U/mL)	2.4	8.0	20.1	24.1	25.6	29.7	33.1	51.0	54.8	0.0	32.6
Max. value (U/mL)	3.7	10.8	23.0	32.1	28.6	39.3	42.7	59.0	64.6	1.2	39.8

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 IgM II 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 CMV IgM 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 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. 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)	14.6	14.6	32.2	21.6	28.4	38.1	59.0	1431	40.7
Standard deviation (U/mL)	0.18	0.17	0.36	0.26	0.35	0.63	1.01	83	0.47
Coefficient of variation (%)	1.2	1.2	1.1	1.2	1.2	1.7	1.7	5.8	1.2
Min. value (U/mL)	13.9	14.0	31.3	20.7	27.5	35.5	55.1	1183	38.9
Max. value (U/mL)	15.0	15.4	33.5	25.0	29.8	39.5	61.2	2313	42.8

*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)	14.6	14.6	32.2	21.6	28.4	38.1	59.0	1431	40.7
Standard deviation (U/mL)	0.25	0.27	0.50	0.98	0.43	0.75	1.11	140	0.64
Coefficient of variation (%)	1.7	1.9	1.6	4.5	1.5	2.0	1.9	9.8	1.6
Min. value (U/mL)	13.9	14.0	31.3	20.7	27.5	35.5	55.1	1183	38.9
Max. value (U/mL)	15.0	15.4	33.5	25.0	29.8	39.5	61.2	2313	42.8

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

15.5. Trueness by recovery test

One set formed of a high- and a low- concentration CMV IgM II 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 CMV IgM II concentrations were analyzed by linear regression. The observed correlation coefficients (r) was 0.9906.

Set 1	Expected concentration, µg/dL	Measured concentration, µg/dL	Recovery (%)
X neat	-	8.35	-
2:1	39.2	43.2	110.1
1:1	55.2	65.6	118.9
1:2	70.8	80.8	113.0
Y neat	-	102.0	-

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 four high-titred samples positive for hCMV IgM. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.7. Analytical and functional sensitivity

Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.654 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® CMV IgM II assay is 2.96 U/mL.
Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® CMV IgM II assay is 2.96 U/mL.

15.8. Diagnostic specificity and sensitivity

Diagnostic performance studies were executed in accordance with Common Specification (CS) published on July 5, 2022.

Diagnostic specificity and sensitivity were assessed by testing 1798 specimens (either serial-panel or single samples) from different populations (subjects sent to the lab for CMV testing, subjects never infected by hCMV, pregnant women, subjects affected by autoimmune diseases, patients affected by other infectious diseases with similar symptoms, patients affected by primary hCMV infection, subjects with past hCMV infection, subjects with long-lasting hCMV 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. 35 specimens were unresolved by the reference methods and therefore were not included in the data analysis.

32 positive, 25 equivocal (reactive) and 1259 negative results were observed out of 1316 samples expected to be negative which were tested during performance evaluation. Diagnostic specificity was 95.67% (1259/1316) when the equivocal zone was applied and both positive and equivocal samples were defined reactive (95% confidence interval: 94.43%-96.64%). Diagnostic specificity was 97.57% (1284/1316) when the equivocal zone was disregarded (95% confidence interval: 96.59-98.27%).

Among the expected negative samples:

Population	Cut-off value	Diagnostic Specificity		95% Confidence Interval
Blood donors	< 18 U/mL	97.83%	(405/414)	95.92% - 98.85%
	< 22 U/mL	99.03%	(410/414)	97.54% - 99.62%
Hospitalized patients	< 18 U/mL	90.58%	(202/223)	86.03% - 93.76%
	< 22 U/mL	92.38%	(206/223)	88.13% - 95.19%
Pregnant women	< 18 U/mL	98.08%	(51/52)	89.88% - 99.66%
	< 22U/mL	98.08%	(51/52)	89.88% - 99.66%
CMV IgM selected samples	< 18 U/mL	91.84%	(90/98)	84.71% - 95.81%
	< 22 U/mL	95.92%	(94/98)	89.97% - 98.40%
Potential cross reactive	< 18 U/mL	96.60%	(511/529)	94.69% - 97.84%
	< 22 U/mL	98.87%	(523/529)	97.55% - 99.48%

425 positive, 16 equivocal (reactive) and 6 negative results were observed out of 447 samples expected to be positive which were tested during performance evaluation. Diagnostic sensitivity in the CMV IgM selected samples was 97.98% (291/297) when the equivocal zone was applied and both positive and equivocal samples were defined reactive (95% confidence interval: 95.66-99.07%). Diagnostic sensitivity was 97.31% (289/297) when the equivocal zone was disregarded 95% confidence interval: 94.78% - 98.63%.

Diagnostic sensitivity in the characterized specimens was 100.0% (150/150) when the equivocal zone was applied and both positive and equivocal samples were defined reactive (95% confidence interval: 97.56-100.0%). Diagnostic sensitivity was 90.67% (136/150) when the equivocal zone was disregarded (95% confidence interval: 84.84-94.80%).

Population	Cut-off value	Diagnostic Specificity		95% Confidence Interval
CMV IgM selected samples	< 18 U/mL	97.98%	(291/297)	95.66% - 99.07%
	< 22 U/mL	97.31%	(289/297)	94.78% - 98.63%
Characterized CMV positive patients	< 18 U/mL	100.0%	(150/150)	97.56% - 100.0%
	< 22 U/mL	90.67%	(136/150)	84.84% - 94.80%

Performance evaluation studies were executed to validate the assay ability to correctly detect acute hCMV infection by testing 123 selected samples, out of which 46 were obtained from subjects with suspected primary hCMV infection and 77 from subjects with diagnosis that excluded primary hCMV infection.

No results below 18 U/mL, no results ranging between 18 and 22 U/mL, and 46 results above 22 U/mL were observed in the population studied of patients with acute hCMV infection. Diagnostic sensitivity was 100% when the equivocal zone was not applied (95% confidence interval: 92.29-100.00%).

No results below 18 U/mL, 13 results ranging between 18 and 22 U/mL, and 64 results above 22 U/mL were observed in the population studied of subjects with long-lasting IgM. Diagnostic sensitivity was 83.12% when the equivocal zone was not applied (95% confidence interval: 72.86-90.70%).

Cut-off value	hCMV IgM - negative samples	Primary hCMV infection	Non-primary hCMV infection
< 18 U/mL	97.1%	0.0%	0.0%
18 - 22 U/mL	2.0%	0.0%	16.9%
≥ 22 U/mL	0.9%	100.0%	83.1%

Six (6) commercially available hCMV IgM seroconversion panels were tested using LIAISON® CMV IgM II and a CE marked reference assay to determine the sensitivity of the assay. The results are summarized in the following table:

Panel ID	LIAISON® CMV IgM II		CE marked reference assay	
	Last day with negative results	First day with positive results	Last day with negative results	First day with positive results
Panel 1	-	1	-	1
Panel 2	29	33	29	33
Panel 3	0	6	0	6
Panel 4	6	9	6	9
Panel 5	27	39	27	39
Panel 6	7	15	7	15

15.9. Performance characteristics of cadaveric blood specimens

Assay characteristics of cadaveric specimens testing was determined by testing, according to PEI validation protocol*, post-mortem specimens collected up to 24 hours after death in comparison to living donor specimens. 20 post-mortem samples were tested as unspiked and spiked at 2 levels: low positive and medium/high positive. The same procedure was performed with the same number of normal human sera from living donors, tested in parallel as reference to compare with post-mortem sample results. The results obtained were analyzed through calculation of percentage difference between mean of living donors results and mean of post-mortem results, at each reactivity level. In this study, the obtained percentage difference was equal or below 1,0% for each of the tested reactivity levels (see table below). Paired t-test analysis were performed between post-mortem and living donors specimens, spiked at low and medium/high positive levels, demonstrating not significantly difference on two groups (p value <0.05).

Repeatability was assessed using one post-mortem and one living donor specimens, spiked up to a low-level of reactivity with a human serum reactive for antibodies to hCMV. Each specimen was assessed in six replicates in the same run. The percent coefficent of variation (CV%) was 3.5% for the cadaveric specimen and 4.1% for the living donor (see table below). The results refer to the group of investigated samples and are not guaranteed specifications, as differences may exist between laboratories and locations.

	Sample	Test results Means (U/mL)	Recovery (%) Post-mortem/Living donors	t-test p value	CV% 6 replicates
Neat	Post-Mortem unspiked	7.10	n.a.	n.a	n.a
	Living donors unspiked	5.00			
Low Positive	Post-Mortem spiked	44.41	0.6	0.629	3.5
	Living donors spiked	44.14			4.1
Medium/high Positive	Post-Mortem spiked	53.84	- 1.0	0.729	n.a
	Living donors spiked	54.38			

* Paul Ehrlich Institute - Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, Anti-HCV-Assays, HBsAg and Anti-HBc Assays for Use with Cadaveric Samples - 08/05/2014

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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