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| **CMV IgG**  |
| **Purpose** | This procedure provides instructions for performing CMV IgG on the DIASORIN LIAISON XL. |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing CMV IgG testing on the DiaSorin Liaison XL. |
| **Principle** | The LIAISON® CMV IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON XL® Analyzer (Model 15970) for the qualitative determination of IgG antibodies to human cytomegalovirus (hCMV) in human serum. It is intended as an aid in the determination of serological status to CMV.The method for qualitative determination of specific IgG to hCMV is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the LIAISON XL® Analyzer. The principal components of the test are magnetic particles (solid phase) coated with hCMV and a conjugate of mouse monoclonal antibody to human IgG linked to an isoluminol derivative. 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 IgG that is already bound to the solid phase. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is induced. The light signal, and the amount of isoluminolantibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of hCMV IgG antibodies present in calibrators, samples or controls. |
| **Clinical Significance** | Human cytomegalovirus (hCMV) is a herpes virus. It is ubiquitous, species-specific, and spread by close human contact. Primary infection may be acquired through different transmission routes and in different periods of life (e.g., congenital, perinatal and post-natal infections). Following primary infection, hCMV enters a latency phase during which the virus can be found in B lymphocytes. Subsequent reactivation of viral replication (secondary infection) may take place concomitantly with changes in the relationship between host and virus. Reinfection with exogenous virus can occur in subjects with deficiency of cellular immunity even when antibodies to hCMV are already present.hCMV infection may be transmitted transplacentally (congenital) or at birth (perinatal). If seronegative women contract primary hCMV infection during pregnancy, the infection is transmitted to the fetus in about 40% of the cases and sequelae may be spontaneous abortion, stillbirth or neonatal malformation. The clinical picture of congenital hCMV infection may be mild to severe and includes psychomotor retardation, deafness, retinochoroiditis, microcephaly, hydrocephalus, cardiac disease, hepatitis, hepatosplenomegaly, or thrombocytopenia. Most individuals (40-90%) acquire primary hCMV infection during childhood or adulthood. Post-natal infections are transmitted through close contact with infected biological fluids (urine, saliva, breast milk, semen, cervical secretions, feces), infected blood products, and, occasionally, organ transplants. In immunocompetent individuals, the clinical picture of post-natal hCMV infection is usually mild or asymptomatic. The most common signs include fever, malaise, and increased serum transaminase levels without jaundice. By contrast in immunocompromised patients (organ transplant recipients, patients with AIDS, lymphoproliferative diseases, or cancer), symptoms may be severe because of disseminated and/or visceral infection, and may include splenomegaly, pneumonia, hemolytic anemia, myocarditis and encephalitis. In these patients the disease may be fatal.The immune response to hCMV involves synthesis of IgM antibodies some weeks after infection by hCMV, and later, IgG antibodies. Levels of IgM to hCMV usually increase for some weeks and decrease slowly thereafter, in four to six months. Occasionally, IgM may circulate for years. IgG antibodies rise gradually and persist for the rest of the host life.The specific IgG assay is useful in distinguishing subjects who have been exposed to the virus from those who have not |
| **Instrument** | DiaSorin LIAISON XLSunquest Method Code: **XL** |
| **Sunquest Test Code** | **CMVG:** Cytomegalovirus Antibodies IgG, part of orderable test: **CMV:** Cytomegalovirus Antibodies IgG/IgM |
| **Sample** | Serum is the only acceptable specimen for this assay collected aseptically by venipuncture. Refer to specimen collection procedures.Grossly hemolyzed, lipemic or particulate samples are not recommendedMinimum volume: 0.2 mLStability: 2-8 °C / 2 days, 30 days at -20 ºC or colderDo not store in self-defrosting freezer.Rejection criteria: Unlabeled tube, plasmaPreparation:Whole blood specimens should be centrifuged as soon as clotted, according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.Clarify samples having particulate matter, turbidity, lipemia, or erythrocyte debrisRemove air bubbles before testingTransfer serum to a properly labeled screw-top tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles. Check for and remove air bubbles before assaying |
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| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | LIAISON® CMV IgG (310740) Integral (100 tests), supplied ready to use, containing magnetic particles, calibrators, diluent and conjugate. | Transfer Pipet capable of delivering 250 µLGlass or polypropylene sample tubes | DiaSorin Liaison XL System |
| **Reagent Integral Preparation** |  |
| **How to prepare and load new integrals**1. Remove from refrigerated storage, maintaining upright orientation
2. Inspect Integral for leakage
3. Mix magnetic particle for 30 seconds
4. Seat test integral in Xcelerator for 30 seconds
5. Gently rotate the magnetic particle vial for 30 seconds
6. Remove new integral sealing flaps slowly
7. Remove all liquid from the surfaces of the membranes to prevent cross-contamination of the reagent vials, blotting with a kim wipe folded in half lengthwise.
8. Open the reagent bay on the analyzer
9. Using a smooth motion, insert the integral into an unoccupied lane in the reagent area until it rests firmly against the docking pins at the rear.

**Note:** if more than one integral of the same reagent is loaded place the newest integral to the right of the old integral. The analyzer will sample from the left integral until empty, then move right. |
| Reagent Integral Storage and Stability:Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles.Stored sealed, the reagents are stable at 2-8°C up to the expiration date. After removing the seals, the Reagent Integral is stable for eight weeks when stored at 2-8°C or on board the LIAISON XL® Analyzer. Record a new expiration date on the integral.Do not freeze. The Reagent Integral must not be used past the expiration date indicated on the kit and reagent integral labels.  |
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| **Special Safety Precautions** | All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents.Specimens should be handled at the BSL 2 level recommended for any potentially infectious human serum or blood specimen.Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands at the end of each assay.Some reagents contain sodium azide as a preservative. Flush drains thoroughly with water after disposal.Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an *overkill* approach. |
| **Calibration** | Assay of calibrators contained in the Reagent Integral allows the Analyzer to recalibrate the stored master curve, as indicated by Frequency Identification transponder (RFID Tag) on the reagent integral label. Refer to the Operator's Manual or LIAISON® XL Quick Guide for calibration instructions.Recalibration is required:* With each new lot of reagents (reagent integral or Starter reagents).
* Every 14 days.
* After servicing the LIAISON XL® Analyzer.
* If quality controls are out of your acceptable range.

Comparable results verify the new reagent lot. Discrepant results must be resolved before the reagent can be used for patient testing. |
| **Analytical Measuring Range (AMR)** | CMV IgG is an FDA-cleared/approved in vitro diagnostic assay that reports the qualitative result based on a predefined cut-off value. Verification of AMR or the cut-off value is not required by CAP or CLIA. The technical range given by DiaSorin is 0.20-10.00 U/mL. |
| **Quality Control** | **LIAISON® CMV IgG Serum Control Set ([REF] 310742)*** Negative control (0.7 mL x 2 vials) containing a barcode label
* Positive control (0.7 mL x 2 vials) containing a barcode label
* Allow controls to reach room temperature prior to use. Invert gently, avoiding bubbles. Return controls to the refrigerator immediately after each use.

**Frequency:** Run 2 levels with each calibration curve and two levels per day. Load the bar-coded control vials into the “T” rack on the Liaison XL.**Stability:** Unopened: Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date. Opened: 8 weeks at 2-8°C between uses. Mark the vials with initials and date of expiration upon opening. Do not use past the expiration date |
| **Procedure** | Refer to the instrument Operating procedure.Strict adherence to the Operator's Manual ensures proper assay performance. **LIAISON**® **XL Analyzer**. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.The Analyzer operations are as follows:1. Dispense calibrators, controls or specimens into the reaction module.2. Dispense coated magnetic particles.3. Dispense 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.Procedural details for the test may be viewed directly from the analyzer's assay definition displays.  |
| **Interpretation/****Results/Alert Values** | The analyzer automatically calculates CMV IgG antibody concentrations expressed as U/mL and grades the results.A **cutoff of 0.7 U/mL** provides the best balance of sensitivity and specificity. An **equivocal range of 0.6 – 0.69 U/mL** was applied to the assay to account for normal measurement imprecision.Results between 0.6 – 0.69 U/mL (***equivocal)*** should be repeat tested. If the result is the same after repeat testing, a second sample should be collected and tested no less than one or two weeks later.**Warning** - When a sample result displays the INVALID RLU flag, repeat the testing.**Note** - *The magnitude of the measured result*, *above the cutoff, is not indicative of the amount of antibody present*.The presence of CMV IgM should also be determined to assess the stage of CMV infection. 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 judgment. Diseases such as Epstein-Barr viral syndrome, toxoplasmosis and hepatitis may cause symptoms similar to CMV infection and must be excluded before confirmation of diagnosis. |

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| **Dilutions** | Do not dilute. See result Reporting. |
| **Reference Intervals** | **<0.6** = Negative, Absence of detectable CMV IgG antibodies indicates that immunity has not been acquired. If exposure to CMV is suspected despite a negative finding, a second sample should be collected and tested no less than one or two weeks later.**0.6 and 0.69** U/mL = Equivocal, a second sample should be collected and tested no less than one or two weeks later.**≥0.7** U/mL = Positive Presence of detectable CMV IgG antibodies. A positive result indicates either recent or past exposure to CMV. |
| Limitations | 1. Do not heat-inactivate sera.
2. The clinical diagnosis must be interpreted with clinical signs and symptoms of the patient. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
3. Results from immunosuppressed patients should be interpreted with caution.Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants.
4. Assay interference due to circulating antibodies against HIV and Hepatitis A, Hepatitis B and Hepatitis C viruses has not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents.
5. The cross-reactivity studies for the LIAISON® CMV IgG assay were designed to evaluate potential interference from IgG immunoglobulins directed against closely-related members of the herpes virus family (EBV, HSV, VZV), from other organisms that may cause symptoms similar to CMV (Hepatitis A virus, Parvovirus B19) and from other conditions that may result from atypical immune system activity [antinuclear antibodies (ANA), rheumatoid factor (RF)]. There was no conclusive evidence of cross-reactivity observed; however due to the limited availability of certain samples, the possibility of cross-reactivity cannot be excluded.

**Interferences:** assay performance was not affected by * hemolysis (at 1000 mg/dL hemoglobin)
* lipemia (at 3000 mg/dL triglycerides)
* icterus (at 20 mg/dL bilirubin)
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| **Result Reporting** | Release results in Sunquest following LIS procedures for OEM. Comments are automatically appended when resulting in OEM or MEM using the XL worksheet.* Results <0.6 U/mL without error messages are reported with the numerical result, and interpreted as Negative. The comment “Absence of detectable CMV IgG antibodies indicates that immunity has not been acquired. If exposure to CMV is suspected despite a negative finding, a second sample should be collected and tested no less than one or two weeks later” is appended.
* Results between 0.6 and 0.69 U/mL must be repeated prior to reporting and are reported with the numerical result, and interpreted as Equivocal. The comment “a second sample should be collected and tested in one or two weeks” appends.
* Results ≥ 0.7 U/mL without error messages are reported with the numerical result, and interpreted as Positive. The comment “Presence of detectable CMV IgG antibodies. A positive result indicates either recent or past exposure to CMV” appends.
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| **Alternate Methods** | * When test performance does not meet quality standards, consult the technical specialist or Medical Director, and refer testing to Mayo Medical Laboratory.
* Order test 80750, CMV Antibody IgG, or test 84420, CMV Antibodies IgG and IgM, and submit 0.5 mL of serum, 0.4 mL minimum for both tests.
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| **References** | 1. LIAISON® CMV IgG (310740) Directions for Use, DiaSorin, Inc, Stillwater, MN 55082, September 2017
2. LIAISON® Control CMV IgG (310741) Directions for Use, DiaSorin, Inc, Stillwater, MN 55082, CMV-G-us.fm, September 2017
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| **Appendices** | Refer to LIAISON® CMV IgG (310740) Directions for Use for specific performance characteristics |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | August 15, 2011 | Initial Version |
|  | Linda Lichty | August 23, 2011 | Added statements for clarification of reporting and QC handling. |
|  | Linda Lichty | October 21, 2016 | Revised sample stability  |
|  | Stephen Gripentrog, Erin Bartos | August 13, 2019 | Updated for DiaSorin Liaison XL |
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