|  |  |  |  |
| --- | --- | --- | --- |
| **CMV IgM** | | | |
| **Purpose** | This procedure provides instructions for performing CMV IgM on the DIASORIN LIAISON XL analyzer in St. Paul laboratory. | | |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing CMV IgM testing on the DiaSorin Liaison XL. | | |
| **Principle** | The method for qualitative determination of specific IgM to hCMV is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the Analyzer. The principal components of the test are magnetic particles (solid phase) coated with hCMV, a buffer of goat IgG to human IgG, and a conjugate of mouse monoclonal antibody to human IgM linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, calibrators, samples or controls are diluted with buffer A, which contains goat IgG to human IgG as an absorbent reagent to curb interference from human IgG specific to hCMV or from rheumatoid factor. During the second incubation, hCMV antibodies present in calibrators, samples or controls bind to the solid phase. During the third incubation, the antibody conjugate reacts with hCMV IgM 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 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 the presence of hCMV IgM antibodies present in calibrators, samples or controls. | | |
| **Clinical Signifiance** | 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.  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. | | |
| **Clinical Significance Cont’d** | 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 thrombocytopoenia. 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. A specific IgM assay is useful in diagnosing acute hCMV infection. However, it is not always possible to distinguish between primary and secondary infection, because reactivation may induce synthesis of IgM in immunocompromised patients | | |
| **Instrument** | DiaSorin LIAISON® XL  Sunquest Method Code: **XL** | | |
| **Sunquest Test Code** | **CMVM**: CMV IgM Antibody. CMVM is a member of the orderable test CMV:  **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 processed 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 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 | | |
|  |  | | |
| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | LIAISON® CMV IgM (310750) Integral (100 tests), supplied ready to use, containing magnetic particles, calibrators, diluent and conjugate. | Polypropylene sample tubes | DiaSorin Liaison XL System |
|  |  | | |
| **Reagent Integral Preparation** | **How to prepare and load new reagent 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 by blotting using 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 tech initials and new expiration date on the integral.Do not freeze.The reagent integral must not be used past the expiration date. | | |
|  |
| **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 Radio 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 IgM 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. DiaSorin stated AMR is 8.00-240.00 AU/mL. | | | |
| **Quality Control** | **The LIAISON® CMV IgM Serum Controls (**[REF] **310752) will adequately control the DiaSorin LIAISON® CMV IgM assay for serum specimens.**   * 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, inverting gently and checking for bubbles prior to testing. Return controls to the refrigerator immediately after each use.   **Frequency:** Run 2 levels each day of use. Load the bar-coded control vials using the QC “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: 16 weeks when stored consistently at 2-8°C between uses.  **Acceptable ranges:**   * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules. * New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot. * Refer to the [Westgard Rules in Chemistry procedure](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) for current Westgard rules in place for each analyte. * **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface. * In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section. * Do not load or release patients until QC is acceptable in Unity Real Time. | | |
| **Procedure** | Refer to the instrument Operating procedure.  Strict adherence to the relevant Analyzer 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 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 buffer A.  3. Incubate.  4. Dispense coated magnetic particles.  5. Dispense specimen diluent.  6. Incubate.  7. Wash with Wash/System liquid.  8. Dispense conjugate into the reaction module.  9. Incubate.  10. Wash with Wash/System liquid.  11. 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. | | |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dilutions** | Do not dilute. See result Reporting. | | | |
| **Reference Intervals** | The analyzer automatically calculates CMV IgM antibody concentrations expressed as AU/mL and grades the results.  <**30 AU/mL** **= Negative**, Absence of detectable CMV IgM antibodies. A negative result, however, does not always rule out acute hCMV infection. The IgM response may not be detectable in the very early stage of the infection or if the patient is immunocompromised. 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.  **Equivocal range of 30.0-34.9 AU/mL** has been applied to account for normal measurement imprecision. Results between 30.0-34.9 AU/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.  **≥35 AU/mL = Positive**  Presence of detectable CMV IgM antibodies. A positive result is generally indicative of acute infection, reactivation or persistent IgM production.  **Note** - *The magnitude of the measured result*, *above the cutoff, is not indicative of the amount of antibody present*.  The presence of CMV IgG should also be determined as it may provide useful information for clinical interpretation of results. 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. | | | |
| Limitations | 1. The test should be performed on serum only. The use of whole blood or plasma specimens has not been established. 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. 4. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of the virus being present. Testing should only be performed on patients with clinical symptoms or when exposure is suspected. 5. The stripping reagent (Buffer A) is able to remove total IgG in a sample up to an amount of 2.8 g/dL. This corresponds to a level in excess of the normal range for serum IgG (0.5-1.8 g/dL). 6. Integrals may not be exchanged between Analyzer types (LIAISON® and LIAISON® XL). Once an Integral has been introduced to a particular Analyzer type, it must always be used on that Analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be conducted between Analyzer types. These must be accomplished on one particular Analyzer type (either LIAISON® or LIAISON® XL).   **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). | | | |
| **Result Reporting** | Released results in Sunquest following LIS procedures for OEM. Comments are automatically appended when resulting in OEM or MEM using the XL worksheet.   * Results <30 AU/mL without error messages are reported with the numerical result, and interpreted as Negative. The comment “Absence of detectable CMV IgM antibodies. A negative result, however, does not always rule out acute hCMV infection. The IgM response may not be detectable in the very early stage of the infection or if the patient is immunocompromised. 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” appends. * Results between 30 and 34.9 AU/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 ≥35 AU/mL without error messages are reported with the numerical result, and interpreted as Positive. The comment “Presence of detectable CMV IgM antibodies. A positive result is generally indicative of acute infection, reactivation or persistent IgM production” appends. | | | |
| **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 87277, CMV Antibody IgM, or test 84420, CMV Antibodies IgG and IgM, and submit 0.5 mL of serum, 0.4 mL minimum for both tests. | | | |
| **References** | 1. LIAISON® CMV IgM (310750) Directions for Use, DiaSorin Inc., Stillwater, MN 55082, January, 2018 2. LIAISON® Control CMV IgM (310752) Directions for Use, DiaSorin Inc., Stillwater, MN 55082 | | | |
| **Appendices** | Refer to LIAISON® CMV IgM (310750) Directions for Use for specific performance characteristics | | | |
|  |  | | | |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | August 15, 2011 | Initial Version |
|  | Linda Lichty | August 22, 2011 | Added statements for clarification of reporting and QC handling. |
|  | Linda Lichty | October 21, 2016 | Revised sample stability |
|  | Erin Bartos | March 3 2017 | Updated catalog number and stability of CMV IgM control. |
|  | Stephen Gripentrog | November 2, 2018 | Removed the use of TPPC and VIRON controls. We will be only be using Liaison controls. |
|  | Stephen Gripentrog | August 13, 2019 | Changed Information for DiaSorin Liaison XL and updated QC reporting for Unity Real Time. |
|  | | | |