CMV IgM

Purpose

This procedure provides instructions for performing CMV IgM on the DIASORIN LIAISON.

Policy Statements

This procedure applies to all laboratory technical staff responsible for performing CMV IgM testing on the DiaSorin Liaison.

Principle

The LIAISON® CMV IgM assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer (Model 15970) for the qualitative determination of IgM antibodies to human cytomegalovirus (hCMV) in human serum. It is intended as an aid in the diagnosis of acute CMV infection. The method for qualititative 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 LIAISON® 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 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 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.

Clinical Significance (cont)

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®, DiaSorin, Inc. Stillwater, MN
Sunquest Method Code: **LIAS**

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 recommended

Minimum volume: 250 µL

Stability: 2-8 °C / 5 days, 45 days at -20 ºC or colder
Do not store in self-defrosting freezer.

Rejection criteria: Unlabeled tube, plasma

Preparation:

- 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 debris
- Remove air bubbles before testing
- Transfer 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. Materials required but not provided: LIAISON® Module (part # 319130) LIAISON® Cleaning Kit (part # 310990) LIAISON® Starter Kit (part # 319102) LIAISON® Light Check (part # 319101) LIAISON® Wash/System Fluid (part # 319100) LIAISON® Waste Bags (part # 9450003) LIAISON® Control CMV IgM (code 310751). (negative, positive)	Transfer Pipet capable of delivering 250 µL Glass or polypropylene sample tubes	DiaSorin Liaison System

Reagent Integral Preparation

1.	Before removing the seals from the containers, gently and carefully shake the Reagent Integral side-to-side. Avoid the formation of foam.
2.	Remove the seal from each container of the Reagent Integral and turn the thumb wheel at the bottom of the magnetic particle container back and forth until the suspension turns brown.
3.	Place the Reagent Integral into the reagent area of the Analyzer with the bar code label facing left and let it stand for 30 minutes before use. The Analyzer automatically stirs and re-suspends the magnetic particles.
4.	Follow the Analyzer Operator's Manual to load the specimens and start the run.

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® Analyzer. Record 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.

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 the bar codes on the Reagent Integral label. Refer to the Operator's Manual or LIAISON® Quick Guide for calibration instructions.

Recalibration in triplicate is required:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- Every 14 days.
- After servicing the LIAISON® Analyzer.
- If quality controls are out of your acceptable range.

Verify new reagent lots before use by testing Liaison CMV IgM Controls, BioRad ToRCH controls, and a weakly positive patient sample (if available). Maintain a rack of previously tested samples for this purpose in the freezer. Document results on the calibration printout.

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.

Quality Control

LIAISON® CMV IgM Controls are used for monitoring substantial reagent failure of the LIAISON® CMV IgM chemiluminescent immunoassay (CLIA).
These controls are not intended to control the 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. Return controls to the refrigerator immediately after each use.

Frequency: Run 2 levels with each calibration curve. Load the bar-coded control vials into the "C" rack on the Liaison, or transfer 220 μL to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability:
Unopened: Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date
Opened: 4 weeks at 2-8°C between uses.

Acceptable ranges: control results within the expected ranges provided on the control vial label validate the test. When control results are outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and samples must be retested.

BIORAD®
Liquichek ToRCH Plus IgM Control, Positive PN# 239
Liquichek ToRCH Plus IgM Control, Negative PN# 228
for use with serum specimens

- Negative control (2.0 mL x 3 vials)
- Positive control (2.0 mL x 3 vials)

Frequency: Two levels once per day of use. Load the bar-coded control vials into the "K" rack on the Liaison, or transfer 220 µL to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability:
Unopened: Store at –20 to -70°C. Stable until the date on vial. Do not use past the expiration date
Opened: 60 days at 2-8°C tightly capped between uses.

Sunquest Control Names:

Negative ToRCH = C-TPMN
Positive ToRCH = C-TPMP

Acceptable ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. Do not report patient results until control results are within expected ranges.

Procedure

Refer to the instrument Operating procedure.
Strict adherence to the Operator's Manual ensures proper assay performance. Each test parameter is identified by the bar codes on the Reagent Integral label. In case of malfunction of the bar code reader, the data can be entered manually. Lot specific information may be retrieved from the accompanying compact disk or by contacting DiaSorin Technical Service.

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.

Interpretation/ Results/Alert Values

The Analyzer automatically calculates CMV IgM antibody concentrations expressed as U/mL and grades the results.

A **cutoff of 30 AU/mL** provides the best balance of sensitivity and specificity.

An **equivocal range of 30.0-34.9 AU/mL** was applied to the assay 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.

Warning - When a sample result displays the exclamation mark **(!) flag**, the result obtained lies below the assay's signal range. With Software V2.0, the sample should be retested and graded negative if the result is still below the signal range upon retest. With Software V2.2, the sample result is not reported, and the sample must be retested.

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.

Dilutions

Do not dilute. See result Reporting.

Reference Intervals

< **30** = 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.

30-34.9 AU/mL = Equivocal

≥**35** AU/mL = Positive
Presence of detectable CMV IgM antibodies. A positive result is generally indicative of acute infection, reactivation or persistent IgM production.

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 IgM assay were designed to evaluate potential interference from IgM 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).

Result Reporting

Review, validate, and tag results and send to Sunquest.
Release results in Sunquest following LIS procedures for OEM. Comments are automatically appended when resulting in OEM or MEM using the LIAS worksheet.

- Results <30 AU/mL without error messages are reported with the numerical result, and interpreted as Negative. Append 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.”
- 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. Append the comment “a second sample should be collected and tested in one or two weeks”
- Results ≥35 AU/mL without error messages are reported with the numerical result, and interpreted as Positive. Append the comment “Presence of detectable CMV IgM antibodies. A positive result is generally indicative of acute infection, reactivation or persistent IgM production.”

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, February 3, 2009
2. LIAISON® Control CMV IgM (310751) Directions for Use, DiaSorin, Inc, Stillwater, MN 55082, CMV-G-us.fm, February 3, 2009

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
1.	Linda Lichty	August 15, 2011	Initial Version
2.	Linda Lichty	August 22, 2011	Added statements for clarification of reporting and QC handling.
3.	Linda Lichty	October 21, 2016	Revised sample stability