en

Immunoassay

REF **CMK0701/CMK0702/CMK0703/CMK0705**

50 tests*1/100 tests*1/100 tests*2/50 tests*2

CMV IgG CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of CMV IgG (specific IgG antibodies to Cytomegalovirus) in human serum or plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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Introduction

CMV (*cytomegalovirus*) is a species of virus that belongs to the viral family known as Herpesviridae. It causes severe and fatal diseases in immune-compromised individuals, including organ transplant recipients and individuals with AIDS. It is also a leading cause of virus-associated birth defects and is associated with atherosclerosis and coronary restenosis.[1]CMV is also the virus most frequently transmitted to a developing fetus and seems to have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality.[2]

Most healthy people who are infected by CMV after birth have no symptoms.[3] Some develop a syndrome similar to infectious mononucleosis or glandular fever, with prolonged fever, and a mild hepatitis.[4] After infection, the virus remains latent in the body for the rest of the person's life. Most infections with CMV are not diagnosed because the virus usually produces few, if any, symptoms and tends to reactivate intermittently without symptoms.

IgG antibodies are produced by the body several weeks after the initial CMV infection and provide protection from primary infections. Levels of IgG rise during the active infection then stabilize as the CMV infection resolves and the virus becomes inactive. After a person has been exposed to CMV, he or she will have some measurable amount of CMV IgG antibody in their blood for the rest of their life. CMV IgG antibody testing can be used, along with IgM testing, to help confirm the presence of a recent or previous CMV infection.

Measurement Principle

This assay is based upon the two-step indirect method. The sample, CMV antigen coated microparticles are combined. IgG antibodies to CMV present in the sample bind to the CMV antigens coated on the microparticles. After washing, Enzyme Conjugate is added. During the incubation, a complex is generated among the solid phase, the CMV IgG within the sample and HRP-conjugated anti-human IgG by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of CMV IgG in the sample.

Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing BSA (bovine serum albumin). Contains sodium azide and ProClin 300® preservatives. Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	50*2
Microparticles Solution	1.2 mL*1	2.3 mL*1	2.3 mL*2	1.2 mL*2
Enzyme Conjugate	5.5 mL*1	11.0 mL*1	11.0 mL*2	5.5 mL*2
Sample Diluent	5.5 mL*1	11.0 mL*1	11.0 mL*2	5.5 mL*2

- Enzyme Conjugate

Horseradish peroxidase labeled mouse anti-human IgG monoclonal antibodies in a Tris-HCl buffer containing bovine serum. Contains ProClin300® preservative.

- Microparticles Solution

Recombinant CMV antigen coated microparticles in PBS (phosphate buffered saline) buffer containing casein. Contains ProClin 300® and sodium azide preservatives.

- Sample Diluent

Tris-HCl buffer containing casein. Contains sodium azide and ProClin 300® preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the CMV IgG calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e. g. Ambient air containing high concentration corrosive gas, such as sodium

hypocritical acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. Ensure the microparticles are re-suspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10 ℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect samples in accordance with correct medical practices.
2. Plasma samples collected in tubes containing EDTA, heparin or sodium citrate have no notable interference to this assay.
3. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit in creased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (un-punctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample tubes on the sample rack, and then add 30 μL of serum or plasma sample which will be automatically diluted by Diluent Universal and mixed well. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the samples to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers Diluent Universal and sample into the reaction vessel and mixed well
  - Aspirates and transfers diluted sample into the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of CMV IgG in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.

- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date (28 days) of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a CMV IgG value exceeding 5000 AU/mL may be diluted manually. Diluent Universal is used to dilute the samples. The concentration of the sample after dilution should not be less than 14 AU/mL. After dilution, multiply the result by the dilution factor. Antibodies to cytomegalovirus are heterogenous. A non-linear dilution behavior is frequently observed.

Measurement Results

The sample test results are determined automatically by the system software. The amount of CMV IgG in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Interpretation of Results

Results obtained with the CMV IgG CLIA Microparticles can be interpreted as follows:
Non-reactive: < 10 AU/mL
Equivocal: 10-14 AU/mL
Reactive: ≥14 AU/mL
A non-reactive result indicates that the specific immunity has not been acquired but cannot rule out the early stage of acute infection. Patients with non-reactive results are still suspected the exposure to cytomegalovirus should be retested within 2 weeks.
A reactive result indicates either early acute infection or past exposure to the pathogen. If it is suspected to be early acute infection, a CMV IgM test or other serological method for detection of additional cytomegalovirus markers, such as a CMV IgG Avidity test, could be performed.
For equivocal result, a second sample should be taken and repeated CMV IgG testing no less than one or two weeks later, or/and using a CMV IgM test to confirm.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the acceptable range. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration maybe necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. However, diagnosis of Toxoplasma infection should not be established on the basis of a single test result as positive or negative for the presence of CMV IgG but should be determined in conjunction with clinical examinations, diagnostic procedures and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. If the patient is immune-compromised or is receiving immune-suppressive therapy (for example, transplant recipients, AIDS patients), the reference value of their IgG antibodies serological detection is limited, and wrong medical explanation may be obtained.
4. A negative result, however, does not always rule out the possibility of cytomegalovirus infection. Because different people have different times from cytomegalovirus infection to produce antibodies, maybe the infections in its very early stage and the patient may be still unable to synthesize enough cytomegalovirus specific IgG. If clinical exposure to cytomegalovirus is suspected despite a negative finding, a second sample should be collected and tested on less than one week later.
5. Samples from neonates, cord blood, pre-transplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.
6. For the samples who have received blood transfusions or other blood products in recent months, the positive result should be given careful analysis.
7. CMV seroprevalence tended to be highest in South America, Africa and Asia and lowest in Western Europe and United States. Within the United States, CMV seroprevalence showed substantial geographic variation as well, differing by as much as 30 percentage points between states, though differences might be explained by variation in the types of populations sampled.[5]
8. This test measures concentrations within the range of 1-5000 AU/mL. If CMV IgG concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up to approximately 50000 AU/mL.

Performance Characteristics

1. Measurement Precision

3 clinical sample panels (1, 2 and 3) and 3 quality control panels (4, 5 and 6) were assayed, using 3 batches of reagents, in replicates of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Lot	Panel Member	n	Mean	Within-run	Total
				%CV	%CV
1	1	80	47.81	4.27	11.74
	2	80	2658.83	3.37	9.39
	3	80	3410.85	3.63	10.26
2	1	80	47.15	3.09	6.30
	2	80	2597.10	3.66	8.73
	3	80	3141.92	4.33	10.59
3	1	80	43.19	2.49	9.00
	2	80	2275.14	4.81	8.98
	3	80	3120.36	5.21	11.32
1	4	80	73.20	6.66	11.66
	5	80	221.40	2.80	10.48
	6	80	374.99	2.13	9.83
2	4	80	71.77	2.68	7.73
	5	80	215.75	2.51	8.23
	6	80	358.43	2.53	7.96
3	4	80	68.77	2.07	4.94
	5	80	205.34	2.79	5.44
	6	80	343.69	3.23	5.04

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Analytical sensitivity represents lowest measurable analyte level that can

be distinguished from zero, is 1 AU/mL.

The study was conducted on 3 reagent batches using 5 human serum-based panels which were prepared at target concentrations. The panel were assayed in replicates of 3 over 4 days for a total of 60 replicates per batch.

3. Analytical Specificity

Cross reaction: This assay is tested to have no cross reactivity with the HSV-1 IgG, HSV-2 IgG, CMV IgG, Rubella IgG, HEV IgG, MP IgG, CP IgG as well as HIV, TP, HCV and HBs antibodies.

Potentially Cross-reactive Samples	Number of Samples	Number of Reactive Samples
HSV-1 IgG	5	0
HSV-2 IgG	5	0
Toxo IgG	10	0
Rubella IgG	10	0
HEV IgG	6	0
HIV antibodies	12	0
TP antibodies	16	0
HCV antibodies	7	0
HBs antibodies	9	0
B19 IgG	7	0
ANA	12	0
Total	99	0

Interference: Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, heparin), bilirubin (up to 20 mg/dL), hemoglobin (up to 1000 mg/dL), triglyceride (up to 3000 mg/dL).

4. Clinical Study

Relative Sensitivity and Relative Specificity: A study was performed where samples were tested using this assay and a reference CMV IgG assay which was already available on the market. Equivocal or inconsistent results after comparison shall be tested by two other reference assays, the result can be determined by at least 3 assays with the same results. Data for relative sensitivity and relative specificity are summarized in the following table.

CMV IgG CLIA Microparticles				
Number of Sample	Relative Sensitivity	Lower 95% CI limit	Relative Specificity	Lower 95% CI limit
893	100%	99.92%	98.95%	96.90%

* CI denotes Confidence Interval

NOTE: A total of 6 samples giving unconfirmed results determined by abovementioned rules were not included in the calculation of relative sensitivity and relative specificity.

Literature References

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