INTERPRETATION OF THE RESULT

IgG Index Value	Result	Interpretation
IgG Index value <0.90	Negative	Indicates absence of prior exposure to cytomegalovirus (<1.1IU/ml)
IgG Index value 0.91-0.99	Grey zone	Sample should be re-tested. (1.1-1.2 IU/ml)
IgG Index value >1.0	Positive	Indicates prior exposure to cytomegalovirus (>1.2IU/ml)

PERFORMANCE CHARACTERISTICS

A total of 86 patient samples were used to evaluate specificity and sensitivity of the test. **ELECTRA™ CMV IgG** test results were compared to a commercial available licensed ELISA kit:

		Reference CLIA			
		N	E	P	Total
ELECTRA™ CMV IgG CLIA	N	45 (D)	0	1 (B)	46
	E	0	1	0	1
	P	1 (C)	0	38 (A)	39
	Total	46	0	39	86

Sensitivity = 97%
Specificity = 98%

Important Note:

1. This assay is a temperature sensitive assay. The best temperature condition for this assay is 37°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.
3. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of Calibrators & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
2. CMV antibody is present in apparently normal subjects in certain populations or geographic groups. A single test is not diagnostic for an active infection. Obtain two specimens at an interval of two weeks and test them at the same time to give more meaningful information.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

BIBLIOGRAPHY

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3. Starr, S.E. and H.M. Friedman. "Human CMV." Chapter 65. In Manual of Clin. Microbiol., 4th ed., Lennett, E.H. et al ed. Am. Soc. Microbiol. pp. 771-719, 1985.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Batch Number / Lot Number
Manufacturer	*In vitro* Diagnostic Medical Device	This side up	Contains sufficient for <n> tests
Use by	Catalogue Number	Do not reuse	

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

An ISO 13485 Certified Company

0821/VER-01

electra™ CMV IgG

Chemiluminescence Assay for Quantitative Detection of CMV IgG antibody in Human Serum
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ CYTOMEGALOVIRUS IgG is intended for the Quantitative detection of IgG antibody to cytomegalovirus infection in human serum. For in Vitro Diagnostic Use only.

INTRODUCTION

Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half of pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness and/or deafness.
Serological tests for detecting the presence of antibody to CMV can provide valuable information regarding the history of previous infection, diagnosis of active or recent infection, as well as in screening blood for transfusions in newborns and immuno-compromised recipients. CLIA CMV IgG is an accurate serologic method to detect CMV antibody for identification of CMV infection.

PRINCIPLE

ELECTRA™ CYTOMEGALOVIRUS IgG CLIA is for use on **ELECTRA™** analyzers. **ELECTRA™ CYTOMEGALOVIRUS IgG** CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalyzed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.
In this assay Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the CMV IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. A subsequent incubation with Anti-human IgG agglutinating sera conjugated with horseradish peroxidase binds to the antigen-antibody complex. Excess enzyme conjugate is washed off, and bound enzyme is detected by adding chemiluminescent substrate and Luminescence is measured in RLU. The intensity of the emitting light is directly proportional to the amount of enzymatic activity of the immunocomplex and hence to the amount of CMV IgG antibodies in the test samples.

MATERIALS & COMPONENTS

Materials provided with the test kits:

1. Coated Microwells: Purified Cytomegalovirus antigen coated wells.
2. Sample Diluent. Ready to use.
3. Negative Calibrator: 0 IU/ml.
4. Positive Calibrator: 6 IU/ml.
5. Positive Calibrator: 18 IU/ml.
6. Negative Control: Range stated on the label
7. Positive Control: Range stated on the label.
8. Wash Buffer Concentrate (20X).
9. Enzyme Conjugate. Ready to use.
10. Cut-off Calibrator: 1.2 IU/ml. CMV G Index = 1.0
11. Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
12. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.

Materials required but not provided:

1. Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. Automated Washer
6. Avidity Buffer
7. **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ CMV IgG** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the color of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).
3. Prepare a working substrate by mixing substrate A and Substrate B in equal volume (1:1 ratio) before addition to the microwells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate- A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate- B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5µl of the test samples, negative control, positive control, and calibrators to 200 µl of sample diluent. Mix well.
3. Dispense 100µl of diluted serum samples, negative control, positive control, and calibrator into the appropriate wells. For the reagent blank, dispense 100µl of sample diluent in A1 well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 45 minutes at 37°C.
4. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
5. Dispense 100µl of enzyme conjugate to each well and incubate for 45 minutes 37°C.
6. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
7. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
8. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (18-25°C) in dark.
9. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA™ Analyzer**. If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **100**µl of sample diluent in A1 Microwell as blank

↓

Add **100** µl of diluted NC, PC, Calibrators and samples into respective wells

↓

Apply plate sealer and incubate for **45** minutes at **37°C**

↓

Wash Microwells **3 times** with diluted wash buffer

↓

Add **100** µl Enzyme conjugate in each well

↓

Apply plate sealer and incubate for **45** minutes at **37°C**

↓

Wash Microwells **3 times** with diluted wash buffer

↓

Add **50** µl of working substrate in each well

↓

Incubate for **10** Minutes at **18-25°C in dark**

↓

Read results on **ELECTRA™** Analyzer exactly at the end of **10** minutes

RUN CRITERIA

The test run may be considered valid provided the following criteria are met:

1. The CMV IgG Index for Negative and Positive Control should be in the range stated on the labels.

AVIDITY TESTING

Avidity is a measure of antigen to antibody binding. Avidity Test helps in discriminating primary infection from secondary infection. Sometimes it is not sufficient to test for IgM antibodies, as the presence of this class may be due to the persistence of IgM antibodies due to past infection or asymptomatic re-infection without risk for the fetus. For this reason it is useful to assay the avidity of IgG antibodies. The presence of low avidity is therefore an indication of recent or current infection. The avidity of IgG antibodies can be assayed with this same kit using an additional Buffer called Avidity Buffer (Cat No. 532010096) which is available on request.

For Procedure and Interpretation of results, kindly refer Pack Insert of Avidity Buffer.

CALCULATIONS

Qualitative Determination of Cytomegalovirus IgG

1. CMV IgG index value can be calculated by dividing the mean absorbance of NC/PC/Sample by absorbance of Cut-Off calibrator (1.2 IU/ml).

$$\text{CMV IgG Index of NC} = \frac{\text{RLU of NC}}{\text{RLU of Cut-Off calibrator}}$$

$$\text{CMV IgG Index of PC} = \frac{\text{RLU of PC}}{\text{RLU of Cut-Off calibrator}}$$

$$\text{CMV IgG Index of sample} = \frac{\text{RLU of Sample}}{\text{RLU of Cut-Off calibrator}}$$

Quantitative Determination of cytomegalovirus IgG

For a quantitative determination of anti-CMV IgG levels of specimens in IU/ml unit, RLU of calibrators are plotted on the Y-axis in graph versus their corresponding anti-CMV IgG concentration 0, 1.2, 6, and 18 IU/ml on the X-axis. The estimates of levels in patient sera are read off the 4 Parameters logistic regression curve using their individual RLU values.

For example:

CMV IgG Values (IU/ml)	RLU's
A	159933
B	5065452
C	6877566
D	8025530