9051 Mira Mesa Blvd, #910094
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# CMV IgG ELISA Kit

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| Name | CMV IgG ELISA Kit |
| INTENDED USE | The CMV IgG ELISA Kit is intended for the detection of IgG antibody to Cytomegalovirus (CMV) in human serum or plasma. |
| SUMMARY AND EXPLANATION | Cytomegalovirus (CMV) is a member of the herpes group of viruses. Most adults and children who catch CMV have no symptoms and are not harmed by the virus. CMV infection is of clinical significance primarily in pregnant women, newborn infants with possible congenital infection, immunosuppressed transplant patients and individuals with AIDS. CMV is so prevalent as over 60% of people catch the infection at some time in their lives. Significant increases in CMV IgG antibody by ELISA suggest recent infection or reactivation of a latent CMV infection. ELISA can detect CMV IgM antibody in both primary CMV infections (93-100%) and in reactivated infection (40%). An IgM response may be reduced or absent in immunocompromised patients with active infection. In transplant patients the CMV infection can be associated with higher morbidity and mortality. |
| PRINCIPLE OF THE TEST | Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. |

| MATERIALS PROVIDED | 96 Tests |
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| Microwells coated with CMV antigen | 12x8x1 |

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| Sample Diluent: 1 bottle (ready to use) | 22 ml |
| Calibrator: 1 Vial (ready to use) | 1ml |
| Positive Control: 1 vial (ready to use) | 1ml |
| Negative Control: 1 vial (ready to use) | 1ml |
| Enzyme conjugate: 1 bottle (ready to use) | 12ml |
| TMB Substrate: 1 bottle (ready to use) | 12ml |
| Stop Solution: 1 bottle (ready to use) | 12ml |
| Wash concentrate 20X: 1 bottle | 25ml |

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450 nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8 C.

2. Keep microwells sealed in a dry bag with desiccants.

3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in

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| | Microbiological and Biomedical Laboratories." 1984.<br>Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.<br>Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.<br>The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.<br>Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water. |
| SPECIMEN COLLECTION AND HANDLING | 1. Collect blood specimens and separate the serum.<br>2. Specimens may be refrigerated at 2–8 C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing. |
| REAGENT PREPARATION | Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C). |
| ASSAY PROCEDURE | Bring all specimens and kit reagents to room temperature (20-25C) and gently mix.<br>Place the desired number of coated strips into the holder Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well.<br>Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.<br>Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel.<br>Dispense 100 l of enzyme conjugate to each well and incubate for 20 minutes at room temperature. |

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| | Remove enzyme conjugate from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel.<br>Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature.<br>Add 100 l of stop solution.<br>Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm. |
| CALCULATION OF RESULTS | Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.<br>Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).<br>Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.<br>Example of typical results:<br>Calibrator mean OD = 0.8<br>Calibrator Factor (CF) = 0.5<br>Cut-off Value = 0.8 x 0.5= 0.400<br>Positive control O.D. = 1.2<br>Ab Index = 1.2 / 0.4 = 3<br>Patient sample O.D. = 1.6<br>Ab Index = 1.6 / 0.4 = 4.0 |
| LIMITATIONS OF THE TEST | The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings and other diagnostic procedures.<br>Lipemic or hemolyzed samples may cause erroneous results. |
| QUALITY CONTROL | The test run may be considered valid provided the following criteria are met:<br>The O.D. of the Calibrator should be greater than 0.250.<br>The Ab index for Negative control should be less than 0.9.<br>The Ab Index for Positive control should be greater than 1.2. |

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## INTERPRETATION

The following is intended as a guide to interpretation of CMV IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

1.1 Detectable antibody to CMV IgG by ELISA.

## PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

180 patient sera were tested by this CMV IgG ELISA and a reference ELISA method. 112 sera were positive and 61 were negative by both methods (96% agreement). The results are summarized below:

| | CMV IgG ELISA | CMV IgG ELISA | CMV IgG ELISA |
|---|---|---|---|
| | + | | Total |
| Reference ELISA Kit + | 112 | 4 | 116 |
| _ | 3 | 61 | 64 |
| Total | 115 | 65 | 180 |

Precision

Intra-Assay Study

| Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation % |
|---|---|---|---|---|
| 1 | 16 | 0.97 | 0.019 | 1.96 |
| 2 | 16 | 0.64 | 0.025 | 3.90 |
| 3 | 16 | 0.09 | 0.007 | 7.77 |

Inter-Assay Study

| Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation % |
|---|---|---|---|---|
| 1 | 10 | 1.39 | 0.14 | 10.07 |
| 2 | 10 | 0.6 | 0.049 | 8.16 |
| 3 | 10 | 0.19 | 0.023 | 12.10 |

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