Chemiluminescence assay

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 immunocompromised 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: 8 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).
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 absorbent sheet to cover the working area.
5. Immediately clean up any spills with sodium hypochlorite.
6. Neutralize acid containing waste before adding hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Do not use kit after the expiry date.
9. Do not mix components of one kit with another.
10. Do not allow liquid from one well to mix with other wells.
11. Always use new tip for each specimen and reagent.
12. Do not let the strips dry in between the steps.
13. Do not eat, drink or smoke in the area where testing is done.

**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.

**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 200µL 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 at 37°C.
6. Wash each well three times by filling approximately 350µL diluted wash buffer & blot dry.
7. Add 50µL 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.

**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.

<table>
<thead>
<tr>
<th>CMV IgG Values (IU/mL)</th>
<th>RLU’s</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>159933</td>
</tr>
<tr>
<td>B</td>
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For example: