Cardiolipin IgG
Enzyme Immunoassay

Cardiolipin (anti-phospholipid) antibodies are frequently observed in patients with systemic lupus erythematosus (SLE), thrombosis and spontaneous abortion. Elevated levels of anticardiolipin antibodies have also been strongly associated with the occurrence of venous and arterial thrombosis, thrombocytopenia, recurrent stroke and recurrent fetal loss.[1,2] Patients who present with these manifestations are known to have an "antiphospholipid syndrome"[2,3,4,5,6].

Cardiolipin antibodies are also found in patients with other autoimmune diseases and in individuals with no apparent autoimmune disease, but who manifest one or more of the clinical complications associated with the presence of anti-phospholipid antibodies in their circulation [3,7,8]. Recurrent pregnancy loss may be a marker for subclinical autoimmune disease, and aCL has been reported as potentially the most sensitive assay to predict fetal distress or death in these patients [9].

Recent studies show that anti-cardiolipin reactions depend both on the phospholipid types and a cofactor (b_2-glycoprotein I). It is also shown that the more specific cofactor-dependent response can be standardized using phosphatidyl serine phospholipid. ***ImmunoWELL*** uses purified cofactor and phosphatidyl serine to produce kits and standardizes each lot assuring consistent cofactor-dependent results.

The ***ImmunoWELL*** Cardiolipin IgG Antibody Test is an EIA that measures IgG anti-Cardiolipin in human serum. The ***ImmunoWELL*** test provides highly reproducible results expressed in units that are standardized against the standards from the Antiphospholipid Standardization Laboratory, University of Louisville. Levels of IgG anti-Cardiolipin antibodies are reported as GPL units.

Expected Results

Although the limit of sensitivity to measure the aCL analyte is 10 GPL units, the cutoff which measures a level of antibody significantly above a normal, asymptomatic population may be higher. The limit was determined by measuring antibody in forty-one normal, asymptomatic subjects. The average titer of aCL was 10.6 GPL units with a range between 0 and 52 GPL units and a standard deviation of 9.1 GPL units. These data support that values above 36 GPL units are significant.

Performance Characteristics

Presumptive positive serum samples were tested using the ***ImmunoWELL*** Cardiolipin IgG Test and the results were compared to an EIA test performed by a clinical reference laboratory. Both tests normalize data against internationally recognized standards (Antiphospholipid Standardization Laboratory) for anti-cardiolipin antibodies. ***ImmunoWELL*** performed essentially the same as the reference test method, showing better than 99% correlation and 100% sensitivity. Using a cut-off level of 36 GPL units, the overall specificity of the ***ImmunoWELL*** Cardiolipin IgG Test is >99%.

Ordering Information

Product Description	Quantity	GenBio Product No.
ImmunoWELL **Cardiolipin IgG Antibody Test**	1 kit / 96 wells	3090
Also available from GenBio		
ImmunoWELL **Cardiolipin IgM Antibody Test**	1 kit / 96 wells	3100

Principle

The ***ImmunoWELL*** Cardiolipin IgG Antibody Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum is added to the antigen coated microtiter wells and allowed to react. After removal of unbound antibodies by washing, horseradish peroxidase-conjugated antihuman IgG antibodies are allowed to react with bound patient antibodies. The bound peroxidase reacts with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS), the chromogenic substrate, developing a color. Finally, the reaction is stopped and the optical density is read with a spectrophotometric microwell reader.

Procedural Summary

1. Prepare Wash Buffer from Wash Buffer Concentrate
2. Predilute each patient specimen and control 1:100 in Specimen Diluent
3. Add 100 µL of Specimen Diluent to specified well as substrate blank
4. Add 100 µL of the prediluted calibrators, patient sera and controls to coated wells and incubate for 60 minutes at room temperature
5. Aspirate wells and wash microwells three times with Wash Buffer
6. Add 100 µL of Conjugate to wells and incubate 30 minutes at room temperature
7. Aspirate microwells and wash wells three times with Wash Buffer
8. Prepare fresh Color Developer
9. Add 100 µL Color Developer to wells and incubate 30 minutes at room temperature
10. Add 100 µL Stop Solution to wells and read results at 405nm

References

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