

ANTIPHOSPHOLIPID SYNDROME: DETECTION AND CLINICAL SIGNIFICANCE OF ANTIPHOSPHATIDYLSERINE ANTIBODIES BY ELISA

Keedy,KJ; Buckner,TR; Taylor,DO; Dier, KJ; O'Sullivan,CA.

REAADS Medical Products, Inc.

Westminster, CO.

Elevated serum levels of antiphospholipid (aPL) antibodies are associated with thrombosis, thrombocytopenia and recurrent abortion (antiphospholipid syndrome). Although cardiolipin is the most commonly used phospholipid antigen in ELISAs, phosphatidylserine, which participates in coagulation, may be a more relevant antigen. An ELISA was developed to determine the clinical relevance of antiphosphatidylserine (aPS) antibodies.

Polystyrene microwells were coated with phosphatidylserine (15µg/ml) in ethanol. Serum samples diluted 1/50 in diluent containing cofactor were incubated in the wells (100µl). After washing, HRP conjugated goat antihuman antibodies were added followed by TMB substrate. The reaction was stopped with 2.5N H₂SO₄ and absorbance read at 450/650nm. Total incubation time: 40 minutes (15,15,10). Results were calculated from a single point calibrator, traceable to a standard preparation, in GPS or MPS (IgG or IgM PhosphatidylSerine) units.

Cut-offs were established at 16 GPS and 22 MPS using 100 healthy blood donors. The assay was linear up to 100 GPS or 80 MPS units ($R^2 \geq 0.98$). Recovery was 80-125%, with intra and inter assay CV's <15%. Specificity was 96% for IgG and 98% for IgM aPS. Sensitivity with a systemic lupus erythematosus (SLE) population was 32% for IgG and 7.5% for IgM. To determine clinical significance, 29 female SLE patients were selected and classified according to clinical manifestations of aPL syndrome: Group I with a history of thrombotic complications and Group II without (control). In Group I, 53% were positive for IgG and 16% for IgM aPS, while in Group II none were positive for IgG and 10% for IgM. Group I showed 25 abortions in 59 pregnancies compared to only 1 abortion in 18 pregnancies in Group II. aPS values in Group I and Group II were statistically different ($p < 0.0001$). Results indicate that IgG aPS antibodies strongly correlate with clinical manifestations of the aPL syndrome.

Presented at 49th Annual Meeting, AACC, Atlanta, GA, 1997. *Clinical Chemistry*, June 1997.