

Differentiating Pathogenic from Non-Pathogenic aPL by a β 2-glycoprotein I Dependency Anti-Cardiolipin ELISA Assay

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Antiphospholipid syndrome (APS) is characterized by recurrent venous or arterial thrombotic events, fetal losses or both. APS can be diagnosed in any race or age, when thrombotic clinical features are accompanied by a positive lupus anticoagulation test or anticardiolipin antibodies (aCL) in two occasions. With the currently available commercial kits, as well as home made assays for detecting aCL antibodies, it is not possible to discriminate non-pathogenic, β 2 GPI independent, infection related antibodies from those of patients with the true APS. We devised an assay that is able to differentiate these two types of antibodies by determining the β 2 GPI requirements to bind in an aCL ELISA. β 2 GPI was purified by perchloric acid precipitation and fixed amounts were used in the dilution solutions of the tested samples that were also tested with no source of β 2 GPI. The ELISA plates were coated with cardiolipin as usual and blocked with a chicken ovalbumin solution. The serum samples had to be highly diluted in order not to have β 2 GPI from the patient serum. The reaction was detected with alkaline phosphate tablets and developed with pNp in diethanolamine buffer. The adapted ELISA aCL assay described here was able to discriminate infectious (syphilis, HCV, dengue fever, HIV and leprosy) and autoimmune (primary APS and SLE related APS). Further testing should be performed to demonstrate that this method consistently differentiates pathogenic antibodies that bind in an aCL ELISA only in the presence of β 2 GPI.

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