Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

# AccuDiag™
# Beta 2 Glycoprotein 1 IgA ELISA

REF 1494-11

IVD See external Label 2°C-8°C Σ 96 Tests

## SUMMARY OF ASSAY PROCEDURE

| Step | Room temperature (20-25° C) | Volume | Incubation time |
|---|---|---|---|
| 1 | Sample dilution 1:101 = 5 µl / 500 µl | | |
| 2 | Washing buffer (3 times) | 350 µl | |
| 3 | Diluted samples, controls & calibrators | 100 µl | 30 minutes |
| 4 | Washing buffer (3 times) | 350 µl | |
| 5 | Enzyme conjugate | 100 µl | 30 minutes |
| 6 | Washing buffer (3 times) | 350 µl | |
| 7 | TMB Chromogenic Substrate | 100 µl | 15 minutes |
| 8 | Stop solution | 100 µl | |
| 9 | Reading OD 450 nm | | |

## INTENDED USE

The DIAGNOSTIC AUTOMATION ß2GP1 IgA Enzyme-linked Immunosorbent Assay (ELISA) is intended for the detection and semiquantitative determination of IgA antibodies to ß2GP1 in human sera or plasma. The results of the assay are to be used as an aid in the diagnosis of certain autoimmune disease thrombotic disorders, anti-phospholipid syndrome, SLE or lupus-like disorders.

## SUMMARY AND EXPLANATION

Cardiolipin autoantibodies (ACA) are described for various autoimmune diseases. The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombocytopenia, in gynaecology they are supposed to cause intrauterine death or recurrent abortion. Furthermore, anti-cardiolipin antibodies have been found in some non-thrombotic neurological disorders like cerebrovascular insufficiency, cerebral ischemia or chorea and in myocardial infarction. (1)
Recent studies have shown that a 50kD serum cofactor is required for anticardiolipin antibodies, to bind to cardiolipin which has been coated onto plastic plates. The cofactor has been identified as ß2-glycoprotein 1 also termed apolipoprotein H. ß2GP1 has been known as an in vitro inhibitor of the intrinsic blood coagulation pathway, ADP-dependent aggregation, and prothrombinase activity of activated platelets. (2~7)
It has become apparent that anticardiolipin antibody from patients with anti-phospholipid syndrome (APS) recognize a modified ß2GP1 structure and not cardiolipin, native ß2GP1 or an epitope structurally defined by both cardiolipin and ß2GP1. (2~6)
Galli et al. (3) and Viard, et al. (8) reported that anti-cardiolipin antibody derived from SLE and APS were directed to the ß2GP1 molecule coated on polystyrene plates. Koike and Matsuura showed conclusively that ß2GP1 is indeed the antigen to which many anticardiolipin antibody patients are actually binding and furthermore showed that the phospholipid merely serves to link the ß2GP1 to the solid phase. (2~9)
ß2GP1 autoantibodies are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune disease, whereas IgG and/or IgA antibodies will be found in progressive stages of manifested autoimmune disorders. IgA antibodies are often associated with IgG antibodies. The determination of IgA antibodies seems to have a greater validity in thrombosis and fetal loss. (10). Indications for determination of anti ß2GP1 antibodies are: SLE, Thrombosis, Thrombocytopenia, Cerebral Ischemia, Chorea, Epilepsy, Recurrent Abortion and Intrauterine Death.

## TEST PRINCIPLE

Purified ß2GP1 antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti ß2GP1 specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgA specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

## STORAGE

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. It is recommended to use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light during storage or usage.

## SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum.
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 of serum sample.

## MATERIALS AND COMPONENTS

**Materials provided with the test kits**

| | | |
|---|---|---|
| 1. | Microwell strips: β2 GP1 antigen coated wells. | 12 x 8 wells |
| 2. | Sample Diluent: White Cap. | 50 ml / bottle |
| 3. | Washing concentrate 20x. | 50 ml / bottle |
| 4. | TMB Chromogenic Substrate: Amber bottle. | 12 ml / bottle |
| 5. | Enzyme conjugate: Red color solution. | 12 ml / bottle |
| 6. | Calibrator set (1:101 prediluted) : 5, 10, 20, 40, 80, 160 SAU. | 1.0 ml / vial |

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7. Control set (1:101 prediluted) : Negative and Positive controls. Ranges are indicated on each label. 1.0 ml / vial
8. Stop solution: 12 ml / bottle

## REAGENT PREPARATION

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to make a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20- 25° C) and gently mix.

## ASSAY PROCEDURE

**1:101 Sample Dilution 100/100/100**
**30/30/15 RT**

1. Place the desired number of coated strips into the holder.
   PRE-WASH Coated Wells - Repeat washing three times with washing buffer.
2. Prepare 1:101 dilution of test samples by adding 5 µl of the sample to 500 µl of Sample Diluent. Mix well.
   Do not dilute 1:101 prediluted Calibrators & Controls.
3. Dispense 100 µl of diluted sera and prediluted calibrators & controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate into each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop solution to stop reaction.
   Make sure there are no air bubbles in each well before reading.
9. Read O.D. at 450 nm with a microwell reader.

## RESULTS

1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator SAU values on the x-axis on a log-log graph paper or log-lin graph.
2. Using the O.D. value of each specimen, determine the concentration from the standard curve.
3. A typical example:

| Calibrator Set | | $\beta_2$GP1 IgA (SAU) | O.D. 450 nm | | O.D. 450 nm Mean | SD | CV % |
|---|---|---|---|---|---|---|---|
| Calibrator | 1 | 5 | 0.089 | 0.085 | 0.087 | 0.003 | 3.251 |
| Calibrator | 2 | 10 | 0.180 | 0.184 | 0.182 | 0.003 | 1.554 |
| Calibrator | 3 | 20 | 0.319 | 0.315 | 0.317 | 0.003 | 0.892 |
| Calibrator | 4 | 40 | 0.573 | 0.589 | 0.581 | 0.011 | 1.947 |
| Calibrator | 5 | 80 | 1.130 | 1.160 | 1.145 | 0.021 | 1.853 |
| Calibrator | 6 | 160 | 2.175 | 2.261 | 2.218 | 0.061 | 2.742 |

## QUALITY CONTROL

1. The negative control and positive control should be run with every batch of samples tested and the concentration must be within the range stated on its label.
2. The O.D. value of blank (Sample Diluent) 0 SAU must be lower than 0.150 and the O.D. value of calibrator 160 SAU must be greater than 0.750.

**Additional controls may be prepared from human serum specimens and kept under -20° C.**

## INTERPRETATION OF RESULTS

**Each laboratory is recommended to establish it own normal range based upon its own techniques, controls, equipments and patient population according to their own established procedures. The followings are a suggestive guideline.**

Negative: < 20 SAU

Low positive: 20 – 40 SAU

Moderate positive: 40 - 70 SAU

High positive: > 70 SAU

A positive result suggests the possibility of certain autoimmune disease thrombolic disorders. A negative result indicates no ß$_2$ GP1 IgA antibody or levels below the detection limit of the assay.

## PERFORMANCE CHARACTERISTICS

**Sensitivity, specificity, and accuracy:**
A total of 75 samples were assayed with the DIAGNOSTIC AUTOMATION ELISA $\beta_2$GP1 IgA (X values) and with a reference ELISA (1) (Y values). The correlation equation is:

$Y = 0.9221 X + 0.5522$ $R^2 = 0.9375$ (n = 75)

| | | Reference ELISA (1) | | |
|---|---|---|---|---|
| | | N | P | Total |
| DAI | N | 32 (D) | 1 (B) | 33 |
| ELISA | P | 3(C) | 39 (A) | 42 |
| ß$_2$GP1 IgA | Total | 35 | 40 | 75 |

Sensitivity = A / (A+B) = 39 / (39 + 1) = 98 %
Specificity = D / (C+D) = 32 / (3 + 32) = 91 %
Accuracy = (A+D) / (A+B+C+D) = (39 + 32) / (39 + 1 + 3 + 32) = 71 / 75 = 95 %.

A second reference ELISA (2) was used to test 3 samples which reference ELISA (1) tested for negative and DIAGNOSTIC AUTOMATION ELISA tested for positive. The results are all positive for the 3 samples. The samples tested for positive with reference ELISA (1) and negative with DIAGNOSTIC AUTOMATION ELISA remains a positive result when it was tested with the reference ELISA (2).

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## Precision:

**Statistic for CV, mean and SD were calculated for each of three samples from the results of 8 determinations in a single run for intra –assay. Inter assay precision was calculated from the result of 8 determinations of 8 different runs.**

| Intra-assay | n | Mean SAU | SD | % CV |
|---|---|---|---|---|
| Serum A | 8 | 14.9 | 0.35 | 2.38 |
| Serum B | 8 | 30.8 | 1.39 | 4.52 |
| Serum C | 8 | 58.9 | 0.99 | 1.68 |

| Inter-assay | n | Mean SAU | SD | % CV |
|---|---|---|---|---|
| Serum A | 8 | 15.6 | 0.38 | 2.4 |
| Serum B | 8 | 31.2 | 1.42 | 4.55 |
| Serum C | 8 | 59.3 | 1.05 | 1.77 |

### INTERFERENCE AND CROSS-REACTIVITY

DIAGNOSTIC AUTOMATION $\beta_2$GP1 IgA test does not cross-react with the following positive samples tested: Rubella, Toxo, CMV, H. pylori, Measles, Mumps, VZV, RF and HSV.

## LIMITATIONS OF THE PROCEDURE

1. Diagnosis cannot be made on the basis of anti ß2 GP1 results alone. These results must be used in conjunction with information from clinical evaluation and other diagnostic procedure.
2. The clinical significance of ß2 GP1 antibodies in diseases other than SLE is currently under investigation.
3. When negative anti ß2 GP1 titers are found in the presence of clinical indications, a lupus anticoagulant, anti-cardiolipin or other additional testing is indicated.
4. It is to be expected that some samples can be anti-cardiolipin positive yet anti ß2 GP1 negative. The anti ß2 GP1 test is a more specific marker of thrombotic risk. The anticardiolipin test can produce false positive results due to cross-reactivity with dsDNA or certain infectious disease antibodies.

## PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found nonreactive for Hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as 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 Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components 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.
5. To prevent injury and chemical burns, avoid contact with skin and eyes or inhalation and ingestion of the following reagents: Enzyme conjugate, TMB chromogenic substrate and Stop solution.

## REFERENCES

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