DRG® Anti-Phosphatidyl serine Ab IgG/IgM (EIA-2580)

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Introduction

Anti-phosphatidyl serine and anti-cardiolipin antibodies along with others, such as the Lupus Anticoagulant (LAC), belong to the family of anti-phospholipid antibodies.

Anti-phosphatidyl serine antibodies are circulating serum antibodies often associated with recurrent arterial and venous thromboembolism, recurrent fetal loss and thrombocytopenia (2).

These symptoms are often present in cases of Systemic Lupus Erythematosus (SLE) and in many other conditions (1). Some studies show > 50 % of SLE patients have one or more classes of anti-phospholipid autoantibodies. The most commonly measured of these autoantibodies are anti-cardiolipin and Lupus Anticoagulant (LAC). Some studies have shown that the measurement of antibodies to other phospholipids including phosphatidyl serine offers clinical benefit, such as increased specificity and clinical sensitivity (1-3). While others have shown that LAC activity is always associated with antibodies against phosphatidyl serine (4,6).

Anti-Phosphatidyl serine autoantibodies can be of any combination of the IgG, IgM and IgA classes. IgG antibodies are the most prevalent class of autoantibody and the class with the greatest clinical correlation (2,5). However, IgM autoantibodies are often found either alone or in association with the IgG class. The clinical relevance of IgA autoantibodies to phosphatidyl serine is still under review.

This kit has the capability to measure all three classes of autoantibody if required.

Patients with anti-phospholipid syndrome may also have autoantibodies which react with other phospholipids. These include antibodies to cardiolipin. An anti-cardiolipin ELISA is also available from DRG.

Principle of the Test

The assay for anti-phosphatidyl serine antibodies is a solid phase immunosorbent assay in which the analyte is indicated by a colour reaction of an enzyme and substrate. The wells are coated with purified antigen.

On adding diluted serum to the wells, any anti-phosphatidyl serine antibody present, binds to the antigen. After incubating and washing away unbound material, horseradish peroxidase conjugated anti-IgG or anti-IgM is added which bind to the anti-phosphatidyl serine antibodies.

Following incubation and washing, the substrate (tetramethyl benzidine) is added to each well. The presence of the {conjugate - anti-phosphatidyl serine antibody - phosphatidyl serine} complex turns the substrate to a dark blue colour. Addition of stop solution turns the colour to yellow.

The colour intensity is proportional to the amount of anti-phosphatidyl serine present in the original sample.

References

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2. Rore, N.S., Dostal-Johnson, D., Ware Branch, M.S., Ware Branch, D. 1990: Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin. Am. J. Obstst. Gynecol. **163**: 575.584.

3. Falcon, C.R., Hoffer, A.M., Forastiero, R.R., Carreras, L.O. 1990: Clinical significance of various ELISA assays for detecting antiphospholipid antibodies. Thromb. Haemostas. **64**: 21-25.
4. Kelsey, P.R., Stevenson, K.J., Poller, L. 1984: The diagnosis of lupus anticoagulants by the activated partial thromboplastin time - the central role of phosphatidyl serine. Throm. Haemostas. **52**, 172-175.
5. Stegnar, M., Bozic, B., Peternel, P., Kveder, T., Vene, N., Rozman, B. 1991: Prevalence of antiphospholipid antibodies in deep vein thrombosis and their relationship to blood coagulation and fibrinolysis. Thromb. Res. **63**: 433-443.
6. Ware-Branch, D., Rote, N.S., Dostal, D.A., Scott, J.R. 1987: Association of lupus anticoagulant with antibody against phosphatidyl serine. Clin. Immunol. Immunopathol. **42**: 63-75.

Precautions

- The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV, HBsAg and HCV by FDA approved procedures. All standards, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
- This kit is for in vitro use only. In the United States, this kit is intended for Research Use Only.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
- Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

Storage and Stability

Store all reagents at 2-8 °C and use before expiry date. Ready to use Wash Buffer is stable for 1 week when stored at 2-8°C. The opened kit should be used within three months.

Unused microtiter strips must always be stored at 2-8 °C in the resealable bag provided. Allow reagents and required number of strips to reach room temperature prior to use.

Contents of the Test

Microtiterstrips 12 x 8 wells
8 wells each, break apart,
coated with phosphatidyl serine purified from bovine spinal cord.

Enzyme Conjugate 2 vials
15 ml each (anti-IgG and anti-IgM),
ready to use.
Conjugated with horseradish peroxidase, pink coloured.

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Calibrators 2 vials
1 ml each, ready for use.

Calibrator	IgG	IgM
(arb.) u/ml	60	60

Calibrators and controls are calibrated against internal standards in arbitrary units.

Control Sera, positive 2 vials
200 µl each for IgG and IgM, dilute 1:100,
(see certificate of analysis for value).

Control Serum, negative 1 vial
200 µl, dilute 1:100,
(see certificate of analysis for value).

Wash Buffer 1 bottle
50 ml, concentrate, 20X
dilute concentrate with distilled water so the final volume is 1 l.
Diluted wash buffer is stable for 1 year at 2 - 8 °C.

Sample Diluent 1 bottle
100 ml, ready to use, blue coloured.

TMB Substrate 1 vial
15 ml, ready to use, TMB solution.

Stop solution 1 bottle
20 ml, ready to use, 0.25 M H_2SO_4.
Caution acid!

Material required but not provided

- Automatic pipettes to dispense 5, 50, 100 and 495 µl (a multichannel pipetting device such as Titertek is suitable for adding reagents to the wells.)
- Distilled water.
- Microtitre plate spectrophotometer (ELISA reader) with 450 nm filter.

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Collection of Specimens and Storage
The specimen should be fresh serum collected by standard procedures. Serum samples showing signs of haemolysis should not be used. If necessary to store sample prior to analysis, it is recommended that, for a period of up to 72 hours, the sample should be stored in a sealed container at 2-8 °C. If longer storage is required, samples should be frozen at - 20 °C. Repeated freeze - thawing should be avoided.

Preparation of Samples and Reagents
All Reagents are ready to use except for the following:

- **Wash Buffer Concentrate**

  Dilute 50 ml of the wash buffer concentrate with distilled water so the final volume is 1 l. Mix well before use. Store this solution at 2-8°C if it is not to be used at once. The diluted wash buffer is stable for 1 week at 2-8°C.
  Important note: Do not allow any detergent to come into contact with the wash buffer, or use wash buffer from other kits as this will affect the assay and produce unreliable results.

- **Controls and serum samples**

  Dilute controls and serum samples 1/100 with Sample Diluent. Prepare fresh control dilutions before each assay run. Vortex all samples and controls before testing.

Assay Procedure
Bring all reagents to room temperature (18-25°C).
Dilute all serum samples and assay controls 1/100 in sample diluent by adding 5 µl to 495 µl sample diluent. Standards do not require dilution.
Pipette **100 µl** of **Calibrator, diluted Controls and diluted Samples** into the wells of the microtiter strips. For Calibrator and Positive Control, use **IgG or IgM.** Duplicate determinations are recommended. To achieve blanking on the ELISA reader a 'no serum' control of 100 µl of sample diluent should be used. For each immunoglobulin class a **separate blank with the respective Enzyme Conjugate** has to be pipetted.

Incubate at room temperature (18 - 25°C) for 30 minutes.

After the first incubation, invert the plate and briskly shake out the well contents. Fill each well with diluted **Wash Buffer** dispensed from a laboratory wash bottle, dispenser or pipette. Repeat this procedure 2 times, then remove excess solution tapping the inverted plated on a paper towel.

Pipet **100 µl** of the **Enzyme Conjugate (anti-IgG or anti-IgM)** into the wells.

Incubate at room temperature (18 - 25°C) for 15 minutes.

Repeat the washing procedure as described in 9.4.

Pipet **100 µl** of **Substrate Solution** into each well.

Incubate for 15 minutes at room temperature (18 - 25°C).

Stop the reaction by adding **50 µl** of **Stop Solution** to each well.

Read the optical density at 450 nm within 15 minutes of stopping using a microplate reader.

Subtract the blank (or mean of blanks) from the optical densities of the standard, controls and patient samples. If the assay was performed in duplicate, the mean of the wells should be taken.

Any sample which gives optical density reading above the value of highest standard should be further diluted with sample diluent buffer and processed again in the test.

Calculation of Results

Results for IgG and IgM anti-phosphatidyl serine antibodies must be calculated separately.

Calculate the mean, blank corrected, absorbance value for all duplicates (samples, controls, calibrators).

Using the following algorithm, calculate the concentration of each of the samples:

$$\text{Conc.} = \frac{\text{Concentration of Calibrator}}{\text{OD of Calibrator}} \times \text{OD sample or control}$$

The calibrator concentrations are:

IgG 60 arb. u/ml

IgM 60 arb. u/ml

Interpretation of results

The following table indicates how the results should be categorized:

	Negative	Low positive	Moderate positive	High positive
IgG u/ml	< 25	25 - 35	36 - 50	> 51
IgM u/ml	< 14	14 - 20	21 - 25	> 26

Typical Example

An example of data which were obtained from a typical Anti-Phosphatidyl Serine ELISA run with calculated results.

		OD	OD	Mean OD	Result (arb.) u/ml
IgG APS	Reagent blank	0.140	0.152	0.146	
	Calibrator	1.977	2.034	2.005	
	Sample 1	0.516	0.515	0.515	11.9
	Sample 2	0.976	1.048	1.012	28.0
IgM APS	Reagent blank	0.200	0.254	0.227	
	Calibrator	1.827	1.879	1.853	
	Sample 1	0.333	0.370	0.352	4.6
	Sample 2	0.646	0.711	0.679	16.7

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Assay Characteristics

Expected Values

* APL = Anti Phospholipid

APL #		Negative (%)	Low positive (%)	Moderate positive (%)	High positive (%)
reactive	IgG	86	6	6	2
	IgM	72	18	8	2
requests	IgG	70	16	4	10
	IgM	92	6	0	2
normals	IgG	98	2	0	0
	IgM	96	4	0	0

Sensitivity

The sensitivity of the assay was established by calculation of the mean of 48 replicates of the zero standards plus two standard deviations which gave values of IgG APS: 1.0 arb. u/ml, IgM APS: 2.8 arb. u/ml.

Specificity

- Anti-Phosphatidyl Serine antibody (APL) reactive
  50 samples were identified as potentially anti-phospholipid antibody positive by a range of test protocols and methods. More than 50 % were found positive within this test.
- APL request
  50 samples were supplied by physicians who suspected anti-phospholipid syndrome. 30 % were positive for IgG and 8 % for IgM anti-phosphatidyl serine antibodies.
- Normals
  From a panel 50 "normal" asymptomatic individuals, 94 % gave negative result while none of the remaining 6 % had a result higher than "low positive".

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Precision

The within batch reproducibility of the anti-phosphatidyl serine assay was based on the analysis of three separate plates as follows:

The between batch reproducibility of the Anti-Phosphatidyl Serine assay was based on the variation of three controls on two routine batches as follows:

Sample	Ig class	Mean (u/ml)	%-CV
1	IgG APS	21.4	4.9
2	IgG APS	2.8	7.4
3	IgG APS	40.1	7.8
1	IgM APS	42.1	10.2
2	IgM APS	33.0	11.1
3	IgM APS	12.8	3.3

Sample	Ig class	Mean	%-CV
1	IgG APS	36.0	4.7
2	IgG APS	16.2	2.5
3	IgG APS	5.3	4.3
1	IgM APS	47.3	3.9
2	IgM APS	33.3	9.5
3	IgM APS	17.8	8.9

Limitations of use

- Grossly hemolyzed, lipemic or microbiologically contaminated samples should not be used.
- A negative result should not be used as a sole criterion to rule out anti-phospholipid syndrome or SLE or other autoimmune disease but must be taken in relation to other clinical observations and diagnostic tests.
- It should be noted that anti-phosphatidyl serine antibodies do occur at low levels in other autoimmune related disorders. Therefore, all other clinical observations and diagnostic tests should be taken into account for clinical diagnosis.

Warranty

Any modification of this test as well as exchange or mixture of any components from different lots might influence the results. In such cases there is no claim for a replacement.