



REF 4050

Juno 16, 2008

INTENDED USE

Anti-Phospholipid screen is used for the separate quantitative determination of IgG and / or IgM antibodies to phospholipides (cardiolipin, phosphatidyl-serine, -inositol, -ethanolamine, phosphatidic acid and beta2 glycoprotein I) in human serum for the diagnosis of anti-phospholipid antibody syndrome (APAS).

APAS is an autoimmune disorder comprising such clinical symptoms like arterial or venous thrombosis, thrombocytopenia and recurrent fetal loss. Primary APAS as well as systemic lupus erythematosus (SLE) are characterized by the appearance of autoantibodies to negatively charged phospholipids (1). Although significance and pathological relevance of phospholipid antibodies are not completely revealed yet, the detection of such autoantibodies is widely established and plays an important role in the diagnostics of systemic autoimmune diseases.

Unlike phospholipid antibodies which appear in some infectious disease patients autoimmune patients exhibit phospholipid antibodies that seem to recognize phospholipids in association with plasma protein cofactors such as beta2 glycoprotein-I (beta2 GP-I) (apolipoprotein H) (2,3). beta2 GP-I, a serum protein with a molecular weight of 50 kDa, affects platelet aggregation and coagulation.

The positively charged fifth domain of beta2 GP-I interacts with negatively charged phospholipids such as Cardiolipin. This interaction results in conformational changes of the protein and the creation of new epitopes apparently recognized by autoimmune phospholipid autoantibodies.

(1) Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young GG, Loizou S and Hughes GRV: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983 11:1211

(2) Galli M, Comfurius P, Maassen C, Hemker HC, DeBaets MHVan Breda-Vriesman PJC, Barbui T, Zwaal RFA, Bevers EM: Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein factor. Lancet 1990 335:1544-1547

(3) McNeil HP, Simpson RJ, Chesterman CN, Krilis SA: Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding factor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990 87:4120-4124

PRINCIPLE OF THE TEST

Anti-Phospholipid screen is an enzyme immunoassay for the quantitative determination of IgG and / or IgM antibodies to negatively charged phospholipids.

The antibodies of the standards, controls and diluted patient samples react with an antigen complex consisting of cardiolipin, phosphatidyl-serine, -inositol, -ethanolamine, phosphatidic acid and the cofactor beta2 GP-I immobilized on the solid phase of microtiter plates. The use of highly purified human beta2 GP-I guarantees the specific binding of autoimmune related phospholipid antibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature, unbound sample components are removed by a wash step.

The bound antibodies react specifically with anti-human-IgG or anti-human-IgM conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature (RT). Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method using a cut-off calibrator is also possible.

Anti-Phospholipid screen

- 96 determinations -



IVD In vitro diagnostic device

Enzyme immunoassay for the determination of IgG and / or IgM antibodies to phospholipids in human serum

Table with 4 columns: REF, Catalogue number, LOT, Batch code. Includes icons for consulting documents, temperature limitation, operating instructions, and biological risk.



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PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.*

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires -20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A	Microtiter plate , 12 breakable strips per 8 wells coated with cardiolipin, phosphatidyl-serine, -inositol, -ethanolamine, phosphatidic acid (bovine) and β ₂ glycoprotein I (human), on each well	1	vacuum sealed with desiccant
Ag	96		
B	Concentrated wash buffer sufficient for 1000 ml solution	100 ml	concentrate capped white
BUF			
WASH	10x		
C	Sample diluent	100 ml	concentrate capped black
DIL			
D	Conjugate containing anti-human-IgG- (sheep) coupled with horseradish peroxidase	15 ml	ready for use capped red
CONJ	G		
E	Conjugate containing anti-human-IgM- (sheep) coupled with HRP	15 ml	ready for use capped green
CONJ	M		
F	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml	ready for use capped blue
SOLN			
TMB			
G	Stop solution 0.25 sulfuric acid	15 ml	ready for use capped yellow
H2SO4	0.25 M		
0 - 4	Calibrators (diluted sera) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each	ready for use capped white
CAL			
P	Positive control (diluted serum) conc.: see leaflet enclosed	1 ml	ready for use capped red
CONTRO	+		

Materials required in addition

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- eppendorf reaction tubes
- glassware
- microplate reader with wavelength for 450nm and 620 nm or 690 nm

Size and storage

Anti-Phospholipid screen has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-Phospholipid screen have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.

For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1+100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (20...25°C) before use. Mix gently without causing foam.
2. Dispense
100 µl calibrators (0 optional) 1 - 4 (quantitative) or
100 µl calibrator 1 (semi-quantitative)
100 µl positive control (P)
100 µl diluted patient samples
 into the respective wells.
3. Incubate **60 min** at room temperature (20...25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D or E) solution to each well.
6. Incubate **30 min** at room temperature (20...25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (F) to each well.
9. Incubate **15 min protected from light** at room temperature (20...25°C).
10. Add **100 µl** of stop solution (G) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

Anti-Phospholipid screen allows both the quantitative (4 + 1 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective anti-phospholipid concentrations on the abscissa, x-axis, (log. scale). Anti-phospholipid concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results are interpreted by calculating the binding index (BI) using **calibrator 1 (10 U/ml)** as **cut-off calibrator**. The BI is the ratio of the OD-value of a sample to the cut-off OD-value (CAL 1).

$$BI = OD_{\text{sample}} / (OD_{\text{calibrator 1}})$$

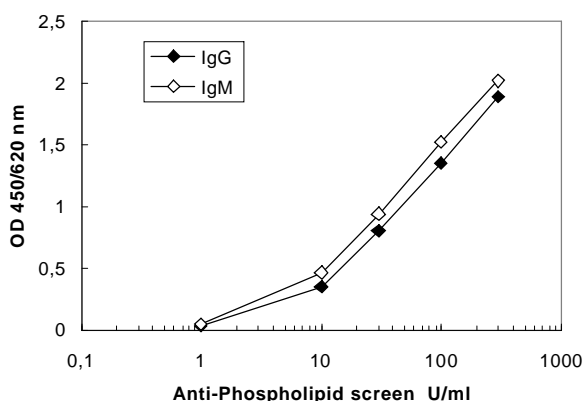
Both evaluation variants of Anti-Phosphatidyl-Serin may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of Typical Assay Results

IgG	OD 1	OD 2	MW OD	U/ml
Calibrator 0	0.038	0.036	0.037	1
Calibrator 1	0.343	0.367	0.355	10
Calibrator 2	0.791	0.816	0.804	30
Calibrator 3	1.341	1.367	1.354	100
Calibrator 4	1.875	1.899	1.887	300
Patient 1	0.661	0.677	0.669	24

IgM	OD 1	OD 2	MW OD	U/ml
Calibrator 0	0.048	0.053	0.050	1
Calibrator 1	0.471	0.467	0.469	10
Calibrator 2	0.936	0.948	0.942	30
Calibrator 3	1.512	1.539	1.525	100
Calibrator 4	2.002	2.034	2.018	300
Patient 1	1.061	1.077	1.069	40

TYPICAL STANDARD CURVES



Specimens with an OD > calibrator 4 should be diluted with phospholipid antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.7
- the mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-Phospholipid screen	U/ml	BI
positive	≥ 10	$\geq 1,0$
negative	< 10	$< 1,0$

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-Phospholipid levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-Phospholipid screen. However, phospholipid autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

No international reference material for this parameter is available so the assay is calibrated in arbitrary units.

Linearity

Selected positive serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

Sensitivity

The analytical sensitivity of this assay was determined at 1.0 U/ml.

Specificity

No cross reactivity to other autoantigens have been found.

Precision

Intra-Assay coefficient of variation (CV): 8-fold determination

sample	IgG			sample	IgM	
	U/ml	CV (%)	U/ml		CV (%)	
1	139.8	5.8	1	130.8	5.6	
2	61.6	6.1	2	71.6	8.4	
3	24.9	4.1	3	46.2	7.2	
4	13.3	7.1	4	22.2	6.0	

Inter-Assay coefficient of variation (CV): 8 different runs of 4-fold determinations:

sample	IgG			sample	IgM	
	U/ml	CV (%)	U/ml		CV (%)	
1	145.3	6.8	1	151.7	7.6	
2	72.3	6.1	2	80.4	5.1	
3	44.0	7.6	3	56.5	6.1	
4	24.8	6.5	4	27.2	4.4	

INCUBATION SCHEME

Anti-Phospholipid screen (4050)

Dilute patients sample	10 µl serum + 1.0 ml sample diluent (C)
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1	Bring all ready for use reagents to room temperature (20...25°C) before use.			
		calibrators	control	sera
2	Pipette calibrators (0 - 4) or calibrator 1 control (P) 1 + 100 prediluted patient sera	100 µl	100 µl	100 µl
3	Incubate 60 minutes at room temperature (20...25°C)			
4	Wash Decant, 3 x 300 µl (made of B)			
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate 30 minutes at room temperature (20...25°C)			
7	Wash Decant, 3 x 300 µl (made of B)			
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light 15 minutes at room temperature (20...25°C)			
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.			

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Source materials derived from bovine material used in the preparation of this kit were tested and found negative for priones. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.