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	See external label	2°C-8°C	Σ=96 tests	Cat # 2560-6

Anti-Phospholipid
Screen IgG/IgM

Cat # 2560–6

PRINCIPLE OF THE PROCEDURE

Anti-Phospholipid Screen (IgG/IgM) is an indirect solid phase enzyme immunometric assay (ELISA). It is designed for the quantitative measurement of IgG or IgM class autoantibodies directed against phospholipids. The microplate is coated with a mixture of highly purified negatively charged phospholipids: Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol and Phosphatidic Acid. Anti-Phospholipid autoantibodies require ß2-Glycoprotein I as a co-factor for binding. The microplate is therefore saturated with human ß2-Glycoprotein I. The microplates can be divided into 12 modules of 8 wells each or can be used completely for 96 determinations. Each well can be separated from the module ("break-away"). The binding of present autoantibodies, formation of the sandwich complexes and enzymatic colour reaction take place during three different reaction phases:

Phase 1:
Calibrators, controls and prediluted patient samples are pipetted into the wells of the microplate. Any present antibodies bind to the inner surface of the wells. After 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

Phase 2:
An anti-human-IgG (or anti-human-IgM) horseradish peroxidase conjugate solution is pipetted into the wells of the microplate to recognise IgG class autoantibodies (or IgM class autoantibodies) bound to the immobilized antigens. After 15 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

Phase 3:
A chromogenic substrate solution containing TMB (3,3`,5,5`-Tetramethylbenzidine) is dispensed into the

wells. During 15 minutes of incubation the colour of the solutions change into blue. Adding 1 M hydrochloric acid as stop solution stops colour development. The solutions colour change into yellow. The amount of colour is directly proportional to the concentration of IgG resp. IgM antibodies present in the original sample. To read the optical density a microplate reader with a 450 nm filter is required. Bi-chromatic measurement with a 600-690 nm reference is recommended.

CLINICAL RELEVANCE

The first study of anti-Phospholipid antibodies began in 1906, when Wasserman introduced a serological test for Syphilis. In 1942, the active component was found to be a phospholipid, which was designated Cardiolipin. In the 1950s it became clear that a number of people had positive tests for syphilis without any evidence of the disease. This phenomenon was referred to as the biological false positive serological test for syphilis. A high prevalence of autoimmune disorders, including systemic lupus erythematosus (SLE) and Sjögrens Syndrome occurred in this group of patients. The presence of circulating anticoagulants in patients with SLE was first documented in 1952 and was associated with increased risk of paradoxical Thrombosis in 1963. The term Lupus anticoagulant (LA), first used in 1972, is clearly a misnomer, because LA is more frequently encountered in patients without lupus and is associated with thrombosis rather than abnormal bleeding. During the last years it became clear that the optimal binding of anti-Phospholipid antibodies is depending on a cofactor termed ß2-Glycoprotein I (apolipoprotein H) (ß2GPI). ß2GPI is a 50-kDa b2-globulin occurring in plasma at a level of 200 µg/ml. It has been found that ß2-Glycoprotein I inhibits the intrinsic coagulation pathway and, therefore, it is involved in the regulation of blood coagulation. ß2GPI is associated in vivo with negatively charged substances, e.g. anionic phospholipids, heparin and lipoproteins. The phospholipid-binding region is located on its fifth domain. Under the acronym "aPL" (anti-Phospholipid antibodies) antibodies against negatively charged phospholipids, such as CL (Cardiolipin), LA (Lupus Anticoagulant), PS (Phosphatidyl Serine), PI (Phosphatidyl Inositol) and PA (Phosphatidic Acid) are summarised. Of these, Cardiolipin is the phospholipid most commonly used as antigen to test for aPL by ELISA. Some Antisera, which bind cardiolipin-coated ELISA plates, can also bind to plates coated with other negatively charged phospholipids, such as Phosphatidyl Serine (PS), Phosphatidyl Inositol and Phosphatidic Acid (PA). Some investigators have suggested that the use of PS in place of cardiolipin in ELISA tests enables more specific diagnosis. These antigens are less commonly used and their additional use can improve the clinical sensitivity in patient samples with suspected APS (Anti-Phospholipid-Syndrome), but they can't replace the measurement of autoantibodies against Cardiolipin.

NORMAL VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-Phospholipid Screen test:

	Anti-Phospholipid-Ab	
	IgG [GPL U/ml]	IgM [MPL U/ml]
Normal:	< 10	< 10
Elevated:	≥ 10	≥ 10

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of anti-Phospholipid antibodies.

SPECIFICITY

The microplate is coated with a mixture of highly purified Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and human ß2-Glycoprotein I. Special coating processes, developed by the manufacturer guarantee for the native immunogenic structure of the phospholipids after immobilization on the solid phase. The anti-Phospholipid Screen test kit is specific for autoantibodies directed against phospholipids or the complex of negatively-charged phospholipids and ß2-Glycoprotein I. No cross-reactivity was observed to anti-DNA antibodies and those types of antibodies occurring in Syphilis.

CALIBRATION

The assay system is calibrated against the internationally recognized reference sera from E.N. Harris, Louisville, since no other international standards are available.

WARNINGS AND PRECAUTIONS

All reagents of this test kit are strictly intended for in vitro use only, except in the United States where it is intended for Research Use Only. Please adhere strictly to the sequence of pipetting steps provided in this protocol. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. All reagents should be stored refrigerated at 2 - 8 °C in their original container. Do not interchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components beyond their expiration dates. Allow all kit components and specimen to reach room temperature prior to use and mix well.
During handling of all kit reagents, controls and serum samples observe the existing legal regulations.
The following precautions should be taken handling potentially infectious materials:
Do not eat, drink or smoke in areas where specimens or kit reagents are handled
Do not pipette by mouth wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
The test kit contains components of human origin which, when tested by FDA-licensed methods, were found negative for hepatitis B surface antigen and for HIV antibody. No known test can guarantee, however, that products derived from human blood will not be infectious. Handle, therefore, all reagents and human blood derivatives, like plasma or serum samples, as if capable of transmitting infection.
Avoid contact with the TMB (3,3`,5,5`-Tetramethyl-benzidine). If TMB comes into contact with skin wash thoroughly with water and soap.
The stop solution contains hydrochloric acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.

MATERIALS SUPPLIED

Package size 96 determ.

Divisible microplate consisting of 12 modules of 8 wells each, 1 coated with a mixture of highly purified phospholipids: Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and saturated with human ß2-Glycoprotein I
Combined calibrators with IgG and IgM class anti-Phospholipid 6 vials, 1.5 ml each antibodies in a PBS/BSA matrix containing:
IgG: 0; 6.3; 12.5; 25; 50; 100 GPL U/ml and IgM: 0; 6.3; 12.5; 25; 50; 100 MPL U/m

anti-Phospholipid controls in a PBS/BSA matrix .... 2 vials, 1.5 ml each
(positive and negative), for the respective concentrations see the enclosed package insert
Anti-Cardiolipin sample buffer, yellow, Concentrate... 1 vial, 20 ml
Enzyme conjugate solution (light red), containing polyclonal .... 1 vial, 15 ml
rabbit anti-h-IgG-IgG, labelled with horseradish peroxidase
Enzyme conjugate solution (light red), containing polyclonal .... 1 vial, 15 ml
rabbit anti-h-IgM-IgG, labelled with horseradish peroxidase
TMB substrate solution.. 1 vial, 15 ml
Stop solution (1 M hydrochloric acid) .... 1 vial, 15 ml
Buffered wash solution, Concentrate .... 1 vial, 20 ml

CONTROLS

A set of two controls is provided with the kit.

TECHNICAL DATA

Sample material:	serum or plasma
Required sample volume:	10 µl of sample to be diluted 1:100 with sample buffer 100 µl prediluted sample per single determination
Total incubation time:	60 minutes at room temperature (20 - 28 °C)
Calibration range:	IgG and IgM: 6.3 - 100 U/ml
Sensitivity:	IgG and IgM: 0.5 U/ml
Storage:	refrigerated at 2 - 8 °C
Shelf life:	12 months after manufacturing or until the expiration date printed on the labels
Package size:	96 tests

MATERIALS REQUIRED

Equipment

- Microplate reader capable for endpoint measurements at 450 nm
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl

Preparation of reagents

- Distilled water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

Optional

- Multi-Chanel Dispenser
- Or repeatable pipet for 100 µl
- Data reduction software

SPECIMEN COLLECTION AND PREPARATION

For determination of anti-Phospholipid serum or plasma are the preferred sample matrixes. All serum and plasma samples are prediluted 1: 100 with sample buffer. Therefore 10 µl of sample may be diluted with 1000 µl of sample buffer. The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation. Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted. Neither Bilirubin nor Hemolysis has significant effect on the procedure.

PREPARATION AND STORAGE OF REAGENTS

All components of this test kit are supplied in a liquid format and ready to use, except the sample buffer and wash buffer. When stored refrigerated at 2 - 8 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels. Remaining modules of the microplate should be stored refrigerated at 2 - 8 °C protected from moisture; store together with desiccant and carefully sealed in the plastic bag.

Preparation of sample buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of buffered wash solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label. **Notes:** Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay. For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

Pipetting and Sample Handling
Use a disposable-tip micropipette to dispense sera and plasma samples. Pipet directly to the bottom of the wells. To avoid carryover contamination changes the tip between samples. Patient samples expected to contain high concentrations should be additionally diluted with sample buffer before. Additional dilutions must be considered during calculation.

IMMUNOASSAY PROCEDURE

Do not interchange components of different lots. All components should be at room temperature before use.
Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 1000 µl of sample buffer in a polystyrene tube. Mix well. Calibrators and controls are ready to use and need not to be diluted.

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and prediluted patient samples in duplicates.

	1	**2**	**3**	**4**	**5**	**6**	
A	SA	SE	P1	P5			
B	SA	SE	P1	P5			

C	SB	SF	P2	P..			SA - SF:	standards A to F
D	SB	SF	P2	P..			P1, P2...	patient sample 1, 2 ...
E	SC	C1	P3				C1:	positive control
F	SC	C1	P3				C2:	negative control
G	SD	C2	P4					
H	SD	C2	P4					

2. For the determination of one class of autoantibodies pipette 100 µl of calibrators, controls and prediluted patient samples into the wells. For determination of both IgG and IgM autoantibodies calibrators, controls and patient samples have to be pipetted in two attempts.
3. Incubate for 30 minutes at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate solution into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules and leave untouched for 5 minutes.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with reference at 600-650 nm is recommended. The developed color is stable for at least 30 minutes. Read optical densities during this time.

CALCULATION OF RESULTS

For anti-Phospholipid Screen IgG and IgM a 4-Parameter-Fit with lin-log co-ordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log co-ordinates are also suitable. **Recommended Lin-Log Plot:** First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight-line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

CALCULATION EXAMPLE

The figures below show typical results for anti-Phospholipid Screen IgG and IgM. These data are intended for illustration only and should not be used to calculate results from another run.

anti-PL	No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
IgG	STA	A 1/B 1	0.051	0.049	0.050	0.3	0.1	0.2	0.0	3
IgG	STB	C 1/D 1	0.163	0.160	0.161	6.4	6.3	6.3	6.3	1
IgG	STC	E 1/F 1	0.310	0.273	0.291	12.8	11.2	12.0	12.5	9
IgG	STD	G 1/H 1	0.603	0.630	0.616	25	26	26	25	3
IgG	STE	A 2/B 2	1.122	1.054	1.088	51	47	49	50	4
IgG	STF	C 2/D 2	1.742	1.787	1.765	98	103	101	100	2
IgM	STA	A 7/B 7	0.022	0.021	0.022	0.2	0.1	0.2	0.0	3
IgM	STB	C 7/D 7	0.211	0.205	0.208	6.1	6.0	6.1	6.3	2

IgM	STC	E 7/F 7	0.465	0.462	0.464	13.0	12.9	13.0	12.5	0
IgM	STD	G 7/H 7	0.788	0.879	0.833	23	26	24	25	8
IgM	STE	A 8/B 8	1.411	1.382	1.397	52	50	51	50	1
IgM	STF	C 8/D 8	1.868	1.852	1.860	101	98	99	100	1

ASSAY CHARACTERISTICS

Sensitivity

The lower detection limits for anti-Phospholipid Screen IgG and IgM were determined at 0.5 U/ml.

Parallelism

In dilution experiments sera with high IgG- and IgM-antibody concentrations were diluted with sample buffer and assayed in the anti-Phospholipid Screen kit. The assay shows linearity over the full measuring range.

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