



REF 4014

March 16^h, 2004

Anti-Cardiolipin Screen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG, IgM and IgA antibodies to Cardiolipin in human serum or plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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INTENDED USE

Anti-Cardiolipin Screen is used for the semi-quantitative determination (screening) of IgG, IgM and IgA antibodies to Cardiolipin in human serum or plasma 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 foetal loss. Primary APAS as well as systemic lupus erythematosus (SLE) are characterized by the appearance of autoantibodies to negatively charged phospholipids including Cardiolipin antibodies (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 Cardiolipin antibodies which appear in some infectious disease patients autoimmune patients exhibit Cardiolipin antibodies that seem to recognize Cardiolipin in association with a plasma protein cofactor. This cofactor has been identified as β_2 glycoprotein-I (β_2 GP-I) (apolipoprotein H) (2,3). β_2 GP-I, a serum protein with a molecular weight of 50 kDa, affects platelet aggregation and coagulation.

The positively charged fifth domain of β_2 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 Cardiolipin 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-Cardiolipin Screen is an enzyme immunoassay for the semi-quantitative determination of IgG, IgM and IgA antibodies to Cardiolipin.

The antibodies of the calibrator and the diluted patient samples react with an antigen complex consisting of Cardiolipin and its cofactor β_2 GP-I immobilized on the solid phase of microtiter plates. The use of highly purified β_2 GP-I guarantees the specific binding of autoimmune related Cardiolipin antibodies of the specimen under investigation. Following an incubation period of 30 min at room temperature, unbound serum components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG, -IgM and -IgA 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 colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 10 min at RT 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 OD values of the unknown patient samples are compared to the OD values of the calibrator.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. 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 (total 96 individual wells) coated with cardiolipin complex	1 vacuum sealed with desiccant
Ag 96		
B	Concentrated wash buffer sufficient for 1000 ml solution	100 ml concentrate capped white
BUF WASH 10 x		
C	Sample diluent	100 ml ready for use capped black
DIL		
D	Conjugate containing anti-human-IgG, anti-human IgM and anti-human IgA (sheep) coupled with HPR	15 ml ready for use capped red
CONJ		
E	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
SOLN TMB		
F	Stop solution 0.25 M sulfuric acid	15 ml ready for use capped yellow
H2SO4 0.25M		
Ca	Calibrator (diluted serum) factor: see leaflet enclosed	1 ml ready for use
CAL		
N	Negative control (diluted serum)	1 ml ready for use
CONTROL		

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-Cardiolipin 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-Cardiolipin 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 per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

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 (18-25°C) before use. Mix gently without causing foam.
2. Dispense
100 µl calibrator (Ca)
100 µl negative control (N)
100 µl diluted patient samples
 into the respective wells.
3. Incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **10 min** protected from light at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) 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

Results are interpreted qualitatively by calculating a cut-off value (A) or semi-quantitatively by calculating the binding index (BI) for each sample (B) on the basis of the cut-off determined:

$$OD_{\text{calibrator}} \times \text{factor} = OD_{\text{cut-off}}$$

The factor for calculation is stated in the control certificate provided in the kit. **The factor value may vary from lot to lot.**

(A) Example for the calculation of the cut-off value:

$$OD_{\text{calibrator}} = 1.214$$

$$\text{factor} = 0.15$$

$$OD_{\text{cut-off}} = 1.214 \times 0.15 = 0.182$$

(B) For the calculation of the binding index (ratio) the following formula should be applied:

$$BI = OD_{\text{sample}} / OD_{\text{cut-off}}$$

Example:

$$OD_{\text{cut-off}} = 0.182$$

$$OD_{\text{sample}} = 0.453$$

$$BI = 0.453 / 0.182 = 2.5$$

This calculation can be performed by the integrated evaluation software of the majority of microplate readers used, too.

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.2
- the mean OD of the calibrator is ≥ 0.7

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-Cardiolipin	BI ratio
negative	< 1,0
positive	≥ 1,0

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-cardiolipin 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-Cardiolipin Screen. However, cardiolipin 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

Anti-Cardiolipin Screen is calibrated according to the reference sera of E.N. Harris, Louisville, USA.

Linearity

Dilutions of selected specimens in Cardiolipin antibody free human serum are determined according to the expected theoretical values with Anti-Cardiolipin Screen.

Analytical sensitivity

The analytical sensitivity of the Anti-Cardiolipin Screen was determined at BI ratio of 0.1.

Functional assay sensitivity

This functional assay sensitivity generally represents that concentration which corresponds to the 10 % (intraassay) and to the 20 % (interassay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of Anti-Cardiolipin Screen, this value is found at a BI ratio of 0.2.

Anti-Cardiolipin Screen values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty. Anti-Cardiolipin Screen concentrations above a BI ratio of 0.2, however, fulfil these criteria and are consequently assessed as valid.

Precision

Intraassay Variation

mean (BI)	CV %
0.5	5.6
1.6	6.0
8.4	5.7

Interassay Variation

mean (BI)	CV %
0.5	6.5
1.9	2.5
4.3	3.8

