



Anti-Cardiolipin Screen

For *In vitro* diagnostic use only

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

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 anti-bodies 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-1 (β_2 GP-1) (apolipoprotein H) (2,3). (β_2 GP-1, a serum protein with a molecular weight of 50 kDa, affects platelet aggregation and coagulation.

The positively charged fifth domain of (β_2 GP-1) 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, Soey ML, Patel BM, Mackworth-Young GG, Lolzou S and Hughes GRV: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983 31:1211
- (2) Galli M, Comfurius P, Maassen C, Hemker HC, DeBaets MHVan Breda-Vriesman PJ, Barbul T, Zwaal RFA, Bevers EM: Anticardiolipin antibodies (ACA) directed not to Gardiolipin but to a plasma protein factor. *Lancet* 1990 335:1544-1547
- (3) McNeil HP, Stimpson RJ, Chesterman CN, Krihs 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-1) immobilized on the solid phase of microtiter plates. The use of highly purified β_2 GP-1 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.

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 $-20^\circ C$.

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 μ l sample + 1.0 ml sample diluent, prior to assay.

The samples may be kept at 2 - 8 $^\circ C$ for up to two days. Long-term storage requires - 20 $^\circ C$.

Kit COMPONENTS

1- Microtiter plate 12 breakable strips per 8 wells (total 96 individual wells) coated with cardiolipin complex.	1 vacuum sealed with desiccant.
2- Concentrated wash buffer sufficient for 1000 ml solution	100 ml concentrate capped white
3- Sample diluent	100 ml ready for use capped black
4- Conjugate Containing anti-human-IgG, anti IgM and anti-human IgA (sheep) coupled with HRP	15 ml ready for use capped red
5- TMB 3,3',5,5'-tetramethylbenzidine in citrate	15 ml ready for use capped blue

buffer containing hydrogen peroxide	
6- Stop solution 0.25 M sulfuric acid	15 ml ready for use capped yellow
7. Calibrators (serum diluted)	1.0 ml each ready for use
Negative control (serum diluted)	1.0 ml ready for use

Materials required but not provided

- micropipette 100-100 0 µl
- micropipette 10-100 µl
- multi-channel pipette or multi-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 tests.

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 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 1 + 100 (v/v), e.g. 10 µl serum + 1 ml sample diluent.

- 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 calibrators
100 µl negative control
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.
5. Add **100 µl** of conjugate 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.
8. Add **100 µl** of TMB solution to each well.
9. Incubate **10 min** protected from light at room temperature (18-25°C).
10. Add **100 µl** of stop solution 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 calibrator x factor = OD 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:

$$\begin{aligned} \text{OD}_{\text{calibrator}} &= 1.214 \\ \text{factor} &= 0.15 \\ \text{OD}_{\text{cut-off}} &= 0.1214 \times 0.15 = 0.182 \end{aligned}$$

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

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

Example

$$\begin{aligned} \text{OD}_{\text{cut-off}} &= 0.182 \\ \text{OD}_{\text{sample}} &= 0.453 \\ \text{BI} &= 0.453 / 0.182 = 2.5 \end{aligned}$$

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

Test validity

The test run is valid if:

- the mean OD of the negative control ≤ 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 Screen	BI Ratio
Negative	<1.0
Positive	≥1.0

It is recommended that each laboratory establishes its own normal and pathological reference ranges, 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

Intra-assay Variation

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

Inter-assay variation

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

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. 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.
- 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.
- 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.

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