

Test Instructions IMTEC-Phosphatidylserine-Antibody Screen

Enzyme Immunoassay for the Quantitative Determination of IgG, IgM and IgA Antibodies against Phosphatidylserine

REF : TC 59027

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different test kits lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

Over the past years, the determination of autoantibodies against phospholipids, detected as anti-cardiolipin antibodies or as lupus anticoagulant (LAC) has become increasingly important due to its association with arterial and venous thrombosis, tendency to abortion, neurological symptoms and signs as migraine and chorea, pulmonary hypertonia as well as thrombocytopenia and hemolytic anemia. Following recently published clinical studies the determination of anti-phosphatidylserine antibodies is an essential criterion in differential diagnosis of patients with anti-cardiolipin antibodies.

The determination of both antibodies should be carried out simultaneously. Anti-phosphatidylserine antibodies (aPs) are strongly associated with the Lupus anticoagulant. Nearly all patients with these multireactive autoantibodies, which are directed against both cardiolipin and phosphatidylserine, suffer from multiple thromboses in a sense of the anti-phospholipid syndrome.

2. Principle of the Test

The test is based on the immobilization of phosphatidylserine in a biological active vesicle like structure to a solid phase (polystyrene) and subsequent binding of the aPs. A better presentation of the antigenic epitope is achieved because of specially purified human β_2 -glycoprotein I (the anti-phospholipid cofactor) was added and the dilution buffer also contains β_2 -glycoprotein I.

The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG, IgM and IgA. After addition of substrate solution, a color stain develops. Its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

3. Materials Provided

-	MTP : phosphatidylserine coated microtiter strips (1x8), breakable	12 strips
-	CAL : standards, ready to use,	750 μ l per vial
1	6.25 U/ml	1 vial each
2	12.5 U/ml	
3	25 U/ml	
4	50 U/ml	
5	100 U/ml	
	(all standards contain sodium azide and are inked according to concentration)	
-	CONTROL - : negative control serum ready to use, contains sodium azide	1 vial 1 ml
-	CONTROL + : positive control serum ready to use, contains sodium azide	1 vial 1 ml
-	BUF WASH 10x : washing buffer, concentrated 10x	1 bottle 50 ml
-	DIL SPE : sample buffer, ready to use	1 bottle 100 ml
-	CONJ a(hum Ig(GAM)):HRP : HRP-Conjugate, anti-human Ig(GAM), ready to use	1 bottle 12 ml
-	SUBS TMB : TMB solution, HRP substrate, ready to use	1 bottle 12 ml
-	SOLN STOP : stopping solution, ready to use, contains sulfuric acid, caution corrosive!	1 bottle 12 ml

4. Preparation of Reagents

Attention! Allow the testkit and all its components to reach room temperature completely before executing it !

Please do not use any polystyrene vessels for handling of HRP conjugates.

In case of running the testkit automatically, it is recommended to use fresh conjugate each time. Please remove traces of old conjugate completely.

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 – 8 °C.

4.2. Preparation of Serum or Plasma

Use serum or plasma freshly collected or freeze samples at –20 °C. Do not use samples, that are repeatedly thawed and frozen. Do not use serum or plasma inactivated by heat treatment at 56 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1:100 with sample buffer [DIL] [SPE] (10 µL sample to 1 mL buffer [DIL] [SPE]).

4.3. Standards, control sera, sample buffer, HRP Conjugate, Stopping Solution and TMB Solution

Standards, control sera, sample buffer, HRP Conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.4. Microtiter Strips

The strips are ready to use. Unused strips should be sealed in the lockable original bag at 2 - 8 °C.

5. Test Procedure

- **Pipette 100 µL serum or plasma dilution** or undiluted standards [CAL] (inked according to rising concentration) or control sera [CONTROL] [+] and [CONTROL] [-] into each well, for blank use sample buffer [DIL] [SPE] instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µl washing buffer per well.
- **Pipette 100 µL of HRP-conjugate** [CONJ] [a(hum Ig(GAM)):HRP] into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µl washing buffer per well.
- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

Calibrate measured absorbance against concentration/units of standards [CAL] (6.25 U/ml, 12.5 U/ml, 25 U/ml, 50 U/ml, 100 U/ml) in semi log. Determine the units of the examined sera or plasma samples from the standard curve directly.

Results above 15 U/ml (cut-off value) are considered positive.

To prove the functionality of the test, the determined value for the positive control serum [CONTROL] [+] is to be expected within the range labeled on the vial. The result of the negative control serum [CONTROL] [-] has to be lower than the cut-off value of the testkit.

Precautions

For in vitro diagnostic use only.

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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