

Test Instructions IMTEC-Sp 100-Antibodies

Enzyme Immunoassay for the quantitative Determination of Anti-Sp 100 Antibodies

REF : TC 66040

Please read the instructions carefully before testing.

Procedural precautions:

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

1. Clinical Use

The primary biliary cirrhosis (PBC) is a chronic inflammatory liver disease. It starts with inflammatory changes in the small and in the medium sized biliary ducts and after slowly developing pathological changes in the tissue, it leads to a complete liver cirrhosis.

Beside the well known marker antibodies of the PBC, the anti-mitochondrial antibodies (AMA) of the type M2, a new antigen has been found in the last few years. It was named Sp 100-antigen after its characteristic immunofluorescence pattern.

Autoantibodies directed against Sp 100-antigen have been detected in 31 % of the patients suffering from PBC. These autoantibodies are found especially frequently (48 %) in patients suffering from PBC but without anti-mitochondrial antibodies.

So the anti-Sp 100 antibody detection improves the diagnosis of PBC because a great number of the AMA-negative patients suffering from PBC will also be picked up. In other autoimmune liver diseases these autoantibodies are not detectable.

Anti-Sp 100 autoantibodies can only be observed in rare cases of patients with rheumatic diseases. The incidence of these autoantibodies in progressive scleroderma amounts to 5 % and in SLE to 1,5 %.

Because of their high specificity the anti-Sp 100 autoantibodies are considered as marker antibodies of PBC.

2. Principle of the Test

The test is based on the binding of a recombinant Sp 100-antigen to the solid phase of microtiter strips and subsequent binding of the anti-Sp 100 antibodies from patient serum. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG. After the addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected autoantibodies.

The test is protected under the German patent number 19624620.

3. Material Provided

-	MTP	: Sp 100 coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
-	CAL	: standards, ready to use	1 vial each 750 µL per vial
1	8	U/mL	
2	40	U/mL	
3	200	U/mL	
4	1000	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 concentrate (10x)	1 bottle 50 mL
-	DIL SPE 5x	: sample buffer concentrate (5x)	1 bottle 22 mL
-	CONJ a(hum IgG):HRP	: HRP-Conjugate, anti-human IgG, 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.

If the test is running 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 Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part sample buffer concentrate [DIL] [SPE] [5x] with 4 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

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

Standards, control sera, 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. Preparation of Samples

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

4.5. Microtiter Strips

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

5. Test Procedure

- **Pipette 100 µL serum or plasma dilution** or standards [CAL], inked according to rising concentration, or control sera [CONTROL] [+] and [CONTROL] [-] into each well, for blank use sample buffer instead of sample 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 IgG):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 absorbances against concentrations/units of standards [CAL] (8 U/mL, 40 U/mL, 200 U/mL, 1000 U/mL) in semi log. Determine the units of the examined samples from the standard curve directly. Results lower than 20 U/mL are negative Results in the range of 20 (cut off value) – 40 U/mL are borderline or slightly positive. Results above 40 U/mL are considered positive.

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

Precautions

For in vitro diagnostics 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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