

Test Instructions IMTEC-Jo-1-Antibodies

Enzyme Immunoassay for the Quantitative Determination of Anti-Jo-1 Antibodies

REF : TC 60025

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

Anti-Jo-1 antibodies are a special case within the group of antinuclear antibodies (ANA) because their corresponding antigen is located only in the cytoplasm. The Jo-1-autoantigen has been identified as the enzyme histidyl-tRNA synthetase.

Antibodies directed against the Jo-1 antigen are especially found in patients with idiopathic inflammatory myopathia. Anti-Jo-1 antibodies can be detected in 33% of patients with primary polymyositis and in 25% of cases with primary dermatositis.

It is remarkable that more than 70% of patients who give positive results in the anti-Jo-1 test are suffering from diffuse fibrosing alveolitis and in some cases from polyarthritis. That is why antibodies directed against the Jo-1 antigen are considered as marker antibodies of a subset of myositis with lung disease.

2. Principle of the Test

The test is based on the absorptive immobilization of recombinant Jo-1 antigen to the solid phase of microtiter plates (polystyrene), and subsequent binding of anti-Jo-1-antibodies. For the detection of anti-Jo-1-antibodies bound to the microtiter plate an antibody directed to human IgG, conjugated with peroxidase, is used. After addition of peroxidase substrate solution, a color stain develops, its intensity is proportional to the concentration and/or the avidity of the anti-Jo-1-antibodies. For anti-Jo-1 antibody quantification the standard sample has been calibrated with the CDC-serum no. 10.

3. Material Provided

-	MTP	: Jo-1 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	12,5 U/ml	
	2	25 U/ml	
	3	50 U/ml	
	4	100 U/ml	
	5	200 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 performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.

4.1. 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.**

4.2. 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.

4.3. 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.

4.4. Preparation of Sera

Use serum samples freshly collected or freeze samples at -20°C. Allow sera to reach room temperature (30 min). Dilute sera 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 dilution** or undiluted standards **CAL**, inked according to concentration, or control sera **CONTROL** **+** and **CONTROL** **-**, into each well, for blanks use sample buffer 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.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **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.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **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

Plot measured absorbances against concentrations/units of standards **CAL** (12.5 U/ml, 25 U/ml, 50 U/ml, 100 U/ml, 200 U/ml) in semilog. Determine the units of the examined sera from the standard curve directly.

Results above 25 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 **CONTROL** **-** has to be lower than the cut-off value of the test.

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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