Test Instructions

Enzyme Immunoassay for the Detection of Anti-U1-snRNP Antibodies

Catalogue-No.: TC 60022

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 kit lots.

1. Clinical Use

Antinuclear antibodies are directed against various ubiquitous antigens in the cell nucleus. The detection of these antibodies plays an important role in the diagnosis of rheumatic diseases. In this regard, antibodies directed against proteins of the spliceosome complex U1-snRNP are of central diagnostic importance. The complex consists of a uridine (U)-rich RNA molecule and different proteins, such as the group of Sm proteins, U1-specific ribonucleoproteins A and C (RNP-A, -C), and the so-called 68 kD protein. Sm proteins also occur in other types of spliceosomal U-RNA (e.g., U2, U4/U6, and U5).

Until now, the detection of RNP autoantibodies was a diagnosis of exclusion. Conventional methods were able to prepare native Sm antigens, but were unable to isolate RNPs alone without Sm proteins. As a result, no definitive determination of the presence or absence of RNP antibodies could be made in patients with Sm-positive serum. Now, the IMTEC assay uses a balanced mixture of the three recombinant RNP proteins A, C and 68-kD, which are bound to the solid phase. Thus allowing the direct and unequivocal detection of RNP antibodies

U1-RNP antibodies are considered to be a diagnostic marker of mixed connective tissue disease (MCTD), which is also referred to as "Sharp's syndrome. Used in this indication, the antibodies achieve a sensitivity of 100% (per definition) and a specificity of 98% in the absence of both Sm and dsDNA antibodies.

Nevertheless autoantibodies directed towards U1-RNP are detectable also in other rheumatoid diseases (SLE: ca. 40%, Sklerodermie: 0-31%, Sjögren Syndrom: 3-15%, Dermatomyositis: 5-14%, chronische Polyarthritis: 10%). But in contrast to MCTD that is characterized by a high anti-nRNP-antibody titers, the other rheumatoid diseases mentioned above, normally display only low activities/titers of anti-nRNP-antibodies.

2. Principle of the Test

The test is based on the immobilisation of recombinant U1-snRNP to a solid phase (polystyrene) and subsequent binding of anti-RNP-antibodies. For the detection of autoantibodies bound to the solid phase, a peroxidase-labeled antibody is used that is directed towards human IgG. After addition of a peroxidase-substrate-solution, a color stain develops. the intensity of that color stain is proportional to the concentration and/or the avidity of the U1-snRNP-antibodies. For antibody quantification the standard sample is calibrated with the CDC-serum num. 4.

3. Material Provided

Material	Quantity
- coated microtiter strips (1 x 8), breakable	12 strips
- standards; ready to use, **1:** 12.5 U/ml, **2:** 25 U/ml, **3:** 50 U/ml, **4:** 100 U/ml, **5:** 200 U/ml (containing sodium azide)	1 vial each á 750 µl
- negative control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- positive control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x), (contains thimerosal)	1 bottle
- sample buffer concentrate (5x), (contains sodium azide)	1 bottle
- conjugate buffer ready to use,	1 bottle 20 ml
- peroxidase conjugate, anti-human IgG, concentrate (100x),	1 vial 200 µl
- peroxidase substrate solution, (TMB), ready to use,	1 bottle 12 ml
- stopping solution, H_2SO_4, ready to use,	1 bottle

4. Preparation of Reagents

Allow the kit to reach room temperature!

4.1 Preparation of Washing Buffer

If any salt has been cristallized inside the bottle it must be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2 Preparation of Sample Buffer

Dilute 1 unit sample buffer concentrate with 4 units distilled water. If any salts has been cristallized it should be resolved before use. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of Standards

The standards are ready to use.

4.4 Preparation of Control Sera

The control sera are ready to use.

4.5 Preparation of Sera

Allow sera to reach room temperature (30 min). Dilute sera 1 : 100 with sample diluent buffer (10 µl serum with 1.0 ml buffer).

4.6 Preparation of Conjugate

The amount of conjugate dilution daily required is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1:100 with conjugate diluent buffer (for one plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining conjugate dilution should be disposed off.

4.7 Preparation of Substrate

The TMB-substrate solution is ready to use. The opened substrate bottle should be closed readily after usage. Store substrate solution at 4 - 8 °C protected from light.

4.8 Microtiter Strips

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

4.9 Stopping Solution

H_2SO_4 (caution!)

5. Test Procedure

1. ***Pipette 100 µl serum dilution*** or (undiluted) standards and control sera into each well, for blanks use ready to use dilution buffer instead of serum dilution, seal wells with adhesive foil
2. ***Incubate for 1 hour*** at room temperature (RT)
3. ***Rinse the wells 3 x*** with a minimum of 200 µl washing buffer per well
4. ***Pipette 100 µl of conjugate dilution*** into each well, seal wells with adhesive foil
5. ***Incubate for 30 minutes*** at RT
6. ***Rinse the wells 3 x*** with a minimum of 200 µl washing buffer per well
7. ***Pipette 100 µl substrate solution*** into each well
8. ***Incubate for 10 min*** at RT in the dark. At room temperatures above 25 °C the substrate incubation time could be shortened, but should never fall short of 5 minutes.
9. ***Pipette 100 µl stopping reagent*** into each well
10. ***Measure at 450 nm within the next 30 min after stopping***

6. Interpretation of Results

Calibrate measured absorbances against concentrations/units of standards (12.5 U/ml, 25 U/ml, 50 U/ml, 100 U/ml, 200 U/ml) in semilog method. Determine the units of the examined sera from the standard curve directly.
Results above 25 U/ml (cut off value) are considered as positive.
To prove the functionality of the test, the determined value for the positive control serum is to be expected within the range labeled on the vial. The result of negative control has to be lower than the cut off value of the test.

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations that were **tested** for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies **with NEGATIVE results**.
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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