

Test Instructions

Enzyme Immunoassay for the Detection of Anti-SS-A/Ro Antibodies

Catalogue-No.: TC 60026

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.

Store reagents at 2-8°C.

1. Clinical Use

Antinuclear antibodies (ANA) are autoantibodies with different specificities which are directed against antigens of the cell nucleus. In general ANA can be divided into antibodies directed against extractable nuclear antigens, nonextractable nuclear antigens and cytoplasmically located antigens. Antibodies against ribonucleoprotein containing nuclear substances like the SS-A/Ro-antigen belong to a group of antibodies which are directed against extractable nuclear antigens, but the SS-A/Ro-antigen are also found in cytoplasm.

In differential diagnosis, autoantibodies directed against SS-A/Ro-antigens are highly specific for Sjogren's syndrome and for systemic lupus erythematosus (SLE).

Especially the immunogenic reactivity of anti-Ro-antibodies to the 60 kD-Ro-protein (Ro60) and the 52 kD-Ro-protein (Ro52) are a valuable tool to discriminate between SLE and primary Sjogren's syndrome respectively.

Anti-SS-A/Ro-antibodies are found in patients suffering from Sjogren's syndrome with a frequency of 85% to 95%. Therefore these antibodies are an important criterion for the classification of this disease.

Anti-Ro52-antibodies are more specific for the primary Sjogren-Syndrome compared to anti-Ro60-antibodies. In over 60% of primary Sjogren-Syndrome Ro52-autoantibodies are occurring isolated (only 5% in SLE).

Anti-SS-A/Ro-antibodies are detectable in 45% to 60% of patients with SLE also. These patients develop a nephritis in significantly more cases than other patients with SLE.

Because of their appearance in asymptomatic patients or in cases of suspicion of the Sjogren-Syndrome without fulfilling the disease criteria especially, Anti-SS-A/Ro-antibodies can also serve as an early diagnostically marker of the syndrome useful to predict the development of the full grown disease.

Antibodies against the SS-A/Ro-antigen are found in 60% of patients with a subacutely cutaneous lupus erythematosus (SCLE). If anti-SS-A/Ro-antibodies are detectable there is an advanced risk of manifestation of SCLE. In such cases a presence of homocysteine C2- (75%) and C4-deficiencies (50%) have been frequently described.

Anti-SS-A/Ro-antibodies have been detected in nearly 100% of newborns with neonatal lupus erythematosus. There is a strong correlation between positive test results for anti-SS-A/Ro-antibodies and the occurrence of a congenital heart block.

Anti-SS-A/Ro autoantibodies are detected in 5% to 7% of patients with rheumatoid arthritis, progressive systemic sclerosis, polymyositis, in 4% of patients with primary biliary cirrhosis, in 3% of patients with a discoid LE and in 0.1% of healthy persons.

Taken together, anti-SS-A/Ro-antibodies are of great importance for prognosis and pathogenesis in patients with SLE or Sjogren's syndrome.

2. Principle of the Test

The test is based on the binding of immobilisation of a mixture of SS-A/Ro-antigens (recombinant and purified by affinity chromatography) to microtiter strips and subsequent binding of anti-SS-A/Ro-antibodies. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG. For antibody quantification the standard is calibrated with the CDC-serum no. 7. After the addition of substrate solution, a color stain develops. Its intensity is proportional to the concentration and/or the avidity of the detected autoantibodies.

3. Material Provided

- coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- 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)	
- 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)	1 bottle 50 ml
- sample buffer concentrate (5x), (contains sodium azide)	1 bottle 22 ml
- Conjugate buffer ready to use	1 bottle 20 ml
- HRP-Conjugate, anti-human IgG, concentrate (100x)	1 vial 200 µl
- HRP substrate (TMB), ready to use	1 bottle 12 ml
- stopping solution, contains H ₂ SO ₄ , ready to use	1 bottle 12 ml

4. Preparation of Reagents

Allow kit to reach room temperature!

4.1. Preparation of Washing Buffer

If any salt has been crystallized 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 Dilution Buffer

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

4.3. Preparation of Standards and Controls

The control sera and standards are ready to use.

4.4. Preparation of Sera

Use serum sera freshly collected or freeze sera at -20 °C. Allow the sera to reach room temperature (30 min). Dilute sera 1 : 100 with sample dilution buffer (10 µl sample with 1 ml buffer).

4.5. Preparation of Conjugate Dilution

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

4.6. Preparation of the Substrate

The TMB-substrate solution is ready to use. The opened substrate bottle should be closed carefully. Store substrate solution at 2 - 8 °C protected from light.

4.7. Microtiter Strips

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

4.8. Stopping Solution

H₂SO₄ (caution !)

5. Test Procedure

- **Pipette 100 µl serum dilution** or (undiluted) standards and control sera into each well, for blanks use sample dilution 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.
- **Pipette 100 µl of conjugate dilution** 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 substrate solution** 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** 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 (12.5 U/ml, 25 U/ml, 50 U/ml, 100 U/ml, 200 U/ml) in semi log. Determine the units of the examined sera from the standard curve directly.

Values below 25 U/ml (cut off) are negative. Results above 25 U/ml are considered positive.

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

Precautions

For in vitro diagnostic use only.

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.



modified 06/2002

IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10
D-13125 Berlin
GERMANY

Tel.: +49(0)30 94 89 36 00

Fax: +49(0)30 94 89 36 15

imtec@mdc-berlin.de www.imtec-berlin.de