

Test Instructions IMTEC-ENA-Screen (cut-off)

Enzyme Immunoassay for the Detection of Anti-ENA Antibodies

REF : TC 70002

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

Antinuclear antibodies (ANA) are autoantibodies with different specificity which are directed against antigens of the cell nucleus. In general, ANA can be divided into antibodies directed against extractable nuclear antigens (ENA), nonextractable nuclear antigens and cytoplasmatically located antigens.

The detection of ANA and ENA-antibodies is important for diagnosis of collagenosis especially of systemic lupus erythematosus (SLE) and the mixed connective tissue disease (MCTD) which is strongly associated with it, and also of other rheumatic diseases.

The nomenclature of extractable nuclear antigens and their antibodies is not standardized. They are either named after the first letter of name of the patient in whom the antibody was first detected (Sm, Ro, La, Jo etc.) or after the disease (SS-A, SS-B: Sjogren`s Syndrome, Scl-70: Scleroderma).

They are also named after the biochemical function of antigens (RNP: Ribo-nucleoprotein).

Since new autoantibodies against previously unknown ENAs are periodically discovered, the base of information on the structure of these antigens and the clinical significance of the antibodies directed against them is constantly growing. This is a continuing process, and the discovery of even more new autoantibodies is to be expected.

Because IMTEC coats its ELISA plates with a cell lysate extract supplemented with additional antigens, it is possible to detect not only the antibodies mentioned in the table, but also other anti-ENA antibodies not listed there because they are extremely rare or because their antigens have not yet been described. Anti-histone antibodies can also be detected.

2. Principle of the Test

The test is based on the covalent binding of mixed ENA to a chemically activated microtiter plate (patent pending) and subsequent binding of the anti-ENA-antibodies from patient serum. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG. After addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

The most important of the already identified ENA antibodies are listed in the table.

autoantibody	relevance	sensitivity
U1-RNP	mixed collagenoses (Sharp-Syndrome/MCTD)	100%
U2-RNP	mixed collagenoses (Sharp-Syndrome/MCTD)	15%
Sm	systemic lupus erythematosus	90%
Ro/SS-A	Sjogren`s-Syndrome	96%
La/SS-B	Sjogren`s-Syndrome	70%
Scl-70	scleroderma	30%
PCNA	systemic lupus erythematosus	3%
Jo-1	polymyositis/dermatomyositis	30-46%
PL-7	polymyositis/dermatomyositis	2-3%
PL-12	polymyositis	2-3%
Mi-2	dermatomyositis	15-31%
Ku	scleroderma-polymyositis-overlap	5-25%
CENP-B	CREST-Syndrome	57-82 %
PM-Scl	scleroderma-polymyositis-overlap	24%

3. Material Provided

- MTP : ENA coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- CONTROL co : cut-off control, ready to use, contains sodium azide	1 vial 2 ml
- 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), contains sodium azide	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. Preparation of Sera

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

4.4. Control Sera, HRP Conjugate, Stopping Solution and TMB Solution

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.**

4.5. 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 control sera **CONTROL** **co**, **CONTROL** **+** and **CONTROL** **-** into each well (see recommended scheme of pipetting), for blanks use ready to 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** with min. 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** with min. 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:

To prove the functionality of the test, the absorbance of the positive control serum **CONTROL** **+** has to be distinctly higher than the absorbance of the cut-off control **CONTROL** **co**. The absorbance of the negative control **CONTROL** **-** has to be lower than the cut-off control **CONTROL** **co** of the test. A patient serum with a measured absorbance that is higher than the absorbance of the cut-off control **CONTROL** **co** possesses an enhanced level of anti-ENA antibodies.

If a serum reacts positively in the cut-off-test the corresponding ENA-antibody should be tested for specific ENAs using the IMTEC-ENA-Profile-test (Cat. No. TC 60033: SS-A/Ro, SS-B/La, SmD1, U1-snRNP, Histone, CENP-B, Scl 70, Jo-1).

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.



last modified: 12/2003

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