Test Instructions
IMTEC-ENA-Profile

Enzyme Immunoassay for the Detection of Anti-ENA-Antibodies (SS-A/Ro, SS-B/La, SmD1, U1-snRNP, histone, CENP-B, Scl 70, Jo-1)

REF : TC 60033

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 specificities which are directed against antigens of the cell nucleus. In general ANA are divided into antibodies directed against extractable nuclear antigens, 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.

Autoantibodies directed against SS-A/Ro-antigen are highly specific in differential diagnosis of the Sjögren's syndrome and of the systemic lupus erythematosus (SLE). Anti-SS-A/Ro-antibodies have been found in patients with the Sjögren's syndrome with a frequency of 85% to 95%. Therefore these antibodies are an important criterion for the classification of this disease.
Anti-SS-A/Ro-antibodies are detectable in 45% to 60% of patients with SLE. These patients develop a nephritis in significantly more cases than other patients with SLE.
Approximately 5% of all patients with SLE are ANA negative. 60% to 80% of these patients possess antibodies against the SS-A/Ro-antigen. This group of patients have developed a Provost syndrome named after the author who first described it.

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 in patients there is an advanced risk of manifestation of SCLE. In such patients a presence of homocygote C2- (75%) and C4-deficiences (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.
Anti-SS-A/Ro-antibodies are of great importance for prognosis and pathogenesis in patients with SLE or Sjögren's syndrome.

Autoantibodies directed against SS-B/La-antigen are especially detected in patients with a Sjögren's syndrome with a frequency of 85%. Such autoantibodies are also detectable in patients with SLE, but only in 10% to 20% of cases.
Anti-SS-B/La-antibodies often occur together with antibodies which are directed against the SS-A/Ro-antigen.
Only in rare cases of other rheumatic diseases antibodies against the SS-B/La-antigen are detectable, in most cases they are not traceable.
Therefore antibodies against the SS-B/La-antigen possess a limited specificity for a Sjögren's-Sicca symptomatic complex.

Autoantibodies to the nuclear Sm antigen were identified as very specific markers for systemic lupus erythematosus (SLE) several years ago. Because they are highly specific, Sm antibodies are an ACR criterion for SLE and are considered to be a pathognomic feature of the disease.
It was recently shown that their sensitivity can be dramatically increased by using a peptide sequence of the D1 protein (aminoacids 83 - 119) as the antigen instead of the entire Sm molecule. When evaluated accordingly, the antibodies were found in up to 70% of the SLE patients tested. This level of sensitivity is associated with a high specificity for SLE also. High concentrations of antibodies were solely observed with SLE and correlate with the level of disease activity.

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. Autoantibodies against RNP can also be detected in patients with various types of rheumatic diseases (SLE: about 40%, scleroderma: 0 to 31%, Sjögren's syndrome: 3 to 15%, dermatomyositis: 5 to 14%, chronic polyarthritis: 10%).
Contrary to MCTD which is characterized by high antibody titre against RNP, the disorders mentioned above show as a rule lower antibody activities/concentrations.

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 68kD, which are bound to the solid phase. Thus allowing the direct and unequivocal detection of RNP antibodies
Anti-histone antibodies are especially detected in 95% of patients with drug-induced lupus erythematosus. Drug-induced anti-histone antibodies are also found in 19% to 30% of patients without symptoms of LE. These patients often develop a drug-induced SLE.

Anti-histone antibodies are detectable in 20% to 50% of SLE patients. These antibodies are also found in approximately 15% to 20% of patients with rheumatoid arthritis, in approximately 20% of patients with juvenile rheumatoid arthritis and with lower frequency in patients with other collagenous diseases.

Anti-centromere antibodies (ACA) are primarily associated with limited forms of progressive systemic sclerosis (PSS), particularly CREST syndrome (calcinosis cutis, Raynaud's phenomenon, esophageal motility dysfunction, sclerodactyly, teleangiectasia); an incidence of 50 - 70% is reported. ACA's appear to signalize a favorable prognosis of PSS. Pulmonary, cardiac and renal manifestations are thus seldom observed in CREST syndrome.
Anti-centromere autoantibodies can be detected in 25% of all patients with Raynaud's phenomenon. This is prognostically significant in these patients, because ACA's are associated with an increased risk of collagenosis. Anti-centromere autoantibodies are detected in 10 - 20% of all patients with primary biliary cirrhosis, but are much less frequent in patients with isolated pulmonary hypertension, SLE or primary Sjögren's syndrome.

Autoantibodies directed against Scl 70 are almost exclusively found in patients with systemic scleroderma with a frequency of 70%. Therefore such antibodies are considered to be marker antibodies of systemic scleroderma. On the other hand autoantibodies against Scl 70 are not detectable in a CREST syndrome which has a better prognosis than systemic scleroderma. However, it is not always possible to differentiate between localized and systemic forms of scleroderma and that is why there is only a restricted possibility to relate sections of autoantibodies to clinical pictures.

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 fibrous 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.

Therefore every rheumatic disease is related to an characteristic ENA-antibody pattern. The IMTEC-ENA-Profile test kit has been developed to detect such antibody profiles.

2. Principle of the Test

The test is based on the covalent binding of extractable nuclear antigens (native and recombinant) to a chemically activated microtiter plate (patent pending) and subsequent binding of the ENA-antibodies. For the detection of the antibodies bound in this way an enzyme-labeled second antibody is used which is directed against human IgG and is coupled with the enzyme peroxidase. For semiquantitative detection of the ENA-antibodies the cut-off control samples have been calibrated with the corresponding CDC-sera.

3. Materials Provided

	color code	+ frame
- MTP : coated microtiter strips, (1 x 12), breakable:		
SS-A/Ro	**green**	1 piece
SS-B/La	**brown**	1 piece
SmD1	**violet**	1 piece
U1-snRNP	**colorless**	1 piece
Histone	**red**	1 piece
CENP-B	**yellow**	1 piece
Scl-70	**blue**	1 piece
Jo-1	**orange**	1 piece

	color code	
- CONTROL co : cut-off-controls, 25 U/ml, ready to use:		
SS-A/Ro	**green**	0.5 ml
SS-B/La	**brown**	0.5 ml
SmD1	**violet**	0.5 ml
U1-snRNP	**colorless**	0.5 ml
Histone	**red**	0.5 ml
CENP-B	**yellow**	0.5 ml
Scl-70	**blue**	0.5 ml
Jo-1	**orange**	0.5 ml

(all cut-off controls are containing sodium azide)

- CONTROL - : negative control serum, ready to use, contains sodium azide — 1 vial, 2.5 ml
- CONTROL + : positive control serum, ready to use, contains sodium azide — 1 vial, 2.5 ml
- BUF WASH 10x : washing buffer concentrate (10x) — 1 bottle, 50 ml
- DIL SPE : sample buffer, ready to use, 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 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. *Preparation of Washing Buffer*

If any salt has been cristallized 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. *Microtiter Strips*

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

4.3. *Sample Buffer, Control sera, HRP Conjugate, Stopping Solution and TMB Solution*

Sample buffer, 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 Sera*

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

5. Test Procedure

- ***Pipette 100 µl serum dilution*** or control sera CONTROL +, CONTROL co and CONTROL - into each well, for blank use sample buffer DIL SPE instead of serum dilution, seal wells with adhesive foil.
- recommended scheme of pipetting:

	1	2	3	4	5	…	12	
A	blank	-	+	co SS-A/Ro	pat. 1	green	pat. 8	SS-A/ Ro
B	blank	-	+	co SS-B/ La	pat. 1	brown	pat. 8	SS-B/ La
C	blank	-	+	co SmD1	pat. 1	violet	pat. 8	SmD1
D	blank	-	+	co U1-snRNP	pat. 1	colorless	pat. 8	U1-snRNP
E	blank	-	+	co histone	pat. 1	red	pat. 8	histone
F	blank	-	+	co CENP-B	pat. 1	yellow	pat. 8	CENP-B
G	blank	-	+	co Scl-70	pat. 1	blue	pat. 8	Scl-70
H	blank	-	+	co Jo-1	pat. 1	orange	pat. 8	Jo-1

- ***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 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

A patient serum with a measured absorbance which is distinctly higher than the absorbance of the cut-off control CONTROL co possesses an enhanced level of antibodies (positive) which are directed against the corresponding ENA-antigen. If a serum reacts positively in the ENA-profile test the concentration of the corresponding antibody should be detected using an IMTEC-test which works quantitatively (Enzyme Immunoassay for the Detection of Anti-SS-A/Ro-, Anti-SS-B/La-, Anti-SmD1-, Anti-U1-snRNP-, Anti-histone-, Anti-CENP-B-, AntiScl-70- or Anti-Jo-1 Antibodies).

To prove the test function on all strips which are coated with different ENA-antigens the absorbance of the positive control serum CONTROL + has to be distinctly higher than the absorbance of the corresponding cut-off control CONTROL co. The negative control CONTROL - has to be lower than the cut-off control CONTROL co in all cases.

Antibody profiles of some rheumatic diseases with a systemic character:

	Ro	La	SmD1	U1-snRNP	histone	Scl-70	Jo-1
chronic polyarthritis	~5%	-	-	-	15-20%	-	-
SLE	~50%	~15%	70%	40%	20-50%	-	-
drug induced SLE	-	-	-	-	95%	-	-
MCTD	~45%	-	-	100%	-	-	-
Sjögren`s Syndrome	~90%	85%	-	-	-	-	-
systemic scleroderma	-	-	-	-		70%	-
polymyositis/ dermatomyositis	-	-	-	-	-	-	25-35%

- = not typical

Precautions

For in vitro diagnostic use only. IVD.

The human control sera and the cut-off controls 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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