Test Instructions
IMTEC-ANA/ENA Profile

Enzyme Immunoassay for the Detection of Antinuclear and ENA-Antibodies

REF : TC 60034

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 (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.
In SLE patients and MCTD patients ANA are detectable at almost 100%. From this follows, that the absence of these antibodies practically excludes a diagnosis of these. A positive detection of ANA is on the other hand no proof for these diseases because ANA are detectable in other diseases, too.
The measurement of autoantibodies against double-stranded DNA (dsDNA) is one important criteria for the diagnosis of systemic lupus erythematosus (SLE). In the remission resp. in an immunosuppressive therapy the titer may dramatically decrease, whereas exacerbations are accompanied by an increase in anti-dsDNA antibodies. So the measured concentration of dsDNA antibodies gives helpful hints for prognosis and therapy of SLE-patients.

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.
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 Sjogren'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 pathognomonic feature of the disease.
It was recently shown that their sensitivity can be dramatically increased by using a peptide sequence of the D1 protein (amino acids 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-snRNP 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 titer 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 68-kD, which are bound to the solid phase. Thus allowing the direct and unequivocal detection of RNP antibodies

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-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.

Therefore every rheumatic disease is related to an characteristic ANA/ENA antibody pattern. The IMTEC-ANA/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 nuclei, of highly purified DNA, of purified histones and of extractable nuclear antigens (native and recombinant) to a chemically activated microtiter plate (patent pending) and subsequent binding of the ANA/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 semi quantitative detection of the ANA/ENA antibodies the cut-off control samples have been calibrated with the corresponding CDC-sera.

3. Materials Provided

		color code	+ frame
-	**MTP** : microtiter strips (1 x 12), breakable coated with:		
	cell nuclei (ANA)	**red**	1 strip
	dsDNA	**white**	1 strip
	SS-A/Ro	**green**	1 strip
	SS-B/La	**brown**	1 strip
	SmD1	**violet**	1 strip
	U1-snRNP	**colorless**	1 strip
	Scl-70	**blue**	1 strip
	histone	**wine-red**	1 strip
-	**CONTROL** **co** : cut-off-controls, ready to use:		
	ANA,40 U/ml	**red**	0.5 ml
	dsDNA antibodies, 25 IU/ml	**white**	0.5 ml
	SS-A/Ro antibodies, 25 U/ml	**green**	0.5 ml
	SS-B/La antibodies, 25 U/ml	**brown**	0.5 ml
	SmD1 antibodies, 25 U/ml	**violet**	0.5 ml
	U1-RNP antibodies, 25 U/ml	**colorless**	0.5 ml
	Scl-70 antibodies, 25 U/ml	**blue**	0.5 ml
	Histone-antibodies, 25 U/ml (containing sodium azide)	**wine-red**	0.5 ml
-	**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.
In case of running the testkit 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 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. *Stopping Solution, Control Sera, Sample Buffer, HRP-Conjugate and TMB Solution*

Stopping solution, Control Sera, sample buffer, HRP-conjugate 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**.

4.3. *Preparation of Sera*

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

4.4. *Microtiter Strips*

The strips are ready to use. Unused strips should be stored in the lockable original plastic 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 DIL SPE instead of serum dilution, seal wells with adhesive foil.
- recommended scheme of pipetting

	1	2	3	4	5	…	12	
A	Blank	-	+	co ANA	Pat. 1	red	Pat. 8	ANA
B	Blank	-	+	co dsDNA	Pat. 1	white	Pat. 8	dsDNA
C	Blank	-	+	co SS-A/Ro	Pat. 1	green	Pat. 8	SS-A/ Ro
D	Blank	-	+	co SS-B/La	Pat. 1	brown	Pat. 8	SS-B/ La
E	Blank	-	+	co SmD1	Pat. 1	violet	Pat. 8	SmD1
F	Blank	-	+	co U1-snRNP	Pat. 1	colorless	Pat. 8	U1-snRNP
G	Blank	-	+	co Scl-70	Pat. 1	blue	Pat. 8	Scl-70
H	Blank	-	+	co histone	Pat. 1	wine-red	Pat. 8	histone

- ***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

To prove the functionality of all strips that are coated with different ANA/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.

In all cases the negative control CONTROL - has to be lower than the cut-off controls CONTROL co.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut-off control sample CONTROL co possesses an enhanced level of specific antibodies directed against the corresponding ANA/ENA-antigens (positive).

If a serum reacts positively in the ANA/ENA-profile test the concentration of the corresponding ANA or anti-ENA should be detected quantitatively using an IMTEC-Enzyme Immunoassay for the Detection of ANA, Anti-dsDNA-, Anti-SS-A/Ro-, Anti-SS-B/La-, Anti-SmD1-, Anti-U1-snRNP-, Anti-Scl-70- or Anti-Histone-Antibodies).

By means of comparing the values of the cut-off controls CONTROL co with these of the patients sera a rough semi quantitative estimation is possible.
Autoantibody profiles of some rheumatic diseases with a systemic character:

	ANA	ds DNA	Ro	La	SmD1	U1-snRNP	Scl-70	histone
chronic polyarthritis	30%	-	~5%	-	-	-	-	15 - 20%
SLE	~ 100%	40 - 90%	~50%	~15%	30%	40%	-	20 - 50%
drug-induced SLE	-	-	-	-	-	-	-	95%
MCTD	~ 100%	-	-	~45%	-	100%	-	-
Sjögren´s Syndrome	60%	-	~90%	-	-	-	-	-
systemic scleroderma	-	-	-	-			70%	-

- = not typical

Precautions

For in vitro diagnostic use only. **IVD**

The human Control Sera an the cut-off Contols 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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