Anti-ENA SLE Profile 2 ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1590-1208-12 G	separate: dsDNA, histones, nucleosomes, nRNP/Sm, Sm, SS-A, SS-B, Scl-70	IgG	Ag-coated microplate wells	12 x 08 (96)

Indications: The ELISA test kit provides a semiquantitative in vitro assay for human autoantibodies of the immunoglobulin class IgG against 8 different antigens (**double-stranded DNA (dsDNA), histones, nucleosomes, nRNP/SM, Sm, SS-A, SS-B, Scl-70)** in serum or plasma for the diagnosis of the Sharp syndrome (MCTD), lupus erythematosus disseminatus, Sjögren's syndrome and progressive systemic sclerosis.

Application: The Anti-ENA SLE Profile 2 ELISA provides parallel determination of antibodies against 8 different nuclear antigens with optional, fully automated processing and objective evaluation of the test results. These antibodies are linked to rheumatic diseases.

Principles of the test: The test kit contains microtiter strips each with 8 reagent wells separately coated with these eight antigens. In the first reaction step, diluted patient samples are incubated with the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 wells in a frame, ready for use: 1. dsDNA, 2. histones, 3. nucleosomes, 4. nRNP/Sm, 5. Sm, 6. SS-A, 7. SS-B, 8. Scl-70	---	12 x 8	STRIPS
2. Calibrator (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
4. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
5. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
6. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
7. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
8. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
9. Test instruction	---	1 booklet	
10. Quality control certificate with factors for calculating the cut-off	---	1 protocol	

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and control:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The calibrator and control of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:201** in sample buffer.
Example: Add 5 µl of sample to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrator and control are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, negative control or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C, protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

A: dsDNA
B: histones
C: nucleosomes
D: nRNP/Sm
E: Sm
F: SS-A
G: SS-B
H: Scl-70

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
B	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
C	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
D	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
E	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
F	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
G	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10
H	C	neg.	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10

The above pipetting protocol is an example of the **semiquantitative determination** of antibodies in 10 patient samples (P 1 to P 10).

Calibrator (C), negative control (neg.) and patient samples have been incubated in the corresponding wells of each microplate strip.
The negative control serum serves as internal control for the reliability of the test procedure. It should be assayed with each test run.

Calculation of results

The extinction value of the calibrator for each individual antigen has to be multiplied by a lot and antigen specific factor. The individual factors are stated on the included quality control certificate. This provides the upper limit of the normal range (cut-off).

Example: Extinction of the calibrator in the reagent well "dsDNA": 1.236
Lot specific factor for "dsDNA": 0.26
Cut-off extinction: 1.236 x 0.26 = 0.321

Values above the recommended cut-off are to be considered as positive, those below as negative.

Besides this qualitative interpretation, a semiquantitative evaluation of results is possible by calculating a ratio according to the following formula:

$$\frac{\textbf{Extinction of patient samples}}{\textbf{Cut - off extinction}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio			Interpretation
<1.0			negative
≥1.0	to	2.0	weak positive
≥2.0	to	5.0	positive
≥5.0			high positive

An indirect immunofluorescence test should always be performed in parallel with the determination of cell nucleus antibodies by ELISA. On the one hand, this provides a check on plausibility as a safeguard against false-positive ELISA results, on the other hand, by using **EUROIMMUN HEp-2 cells**, and in particular **in combination with frozen sections of primate liver**, immunofluorescence permits the detection of a wider range of cell nucleus antibodies, as not all cell nucleus antigens are presently available in the ELISA substrate.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: The Anti-ENA SLE Profile 2 ELISA (IgG) is calibrated with a mixed serum which contains anti-bodies against the antigens used in this test system. An individual cut-off extinction is calculated for each antigen with the aid of a lot specific factor. Results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the negative control must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigens: The microplate wells were separately coated with the following antigens:

dsDNA: The antigen substrate consists of dsDNA which is complexed with nucleosomes (NcX) and coupled to the solid phase.

Histones: A mixture of individually purified histone types H1, H2A, H2B, H3 and H4 isolated from calf thymus.
Histones are basic DNA-associated proteins with molecular weights from 11.2 kDa to 21.5 kDa. Their function is to stabilise the DNA double helix and also they might play a role in gene regulation mechanisms. Five distinct histone types exist: H1, H2A, H2B, H3, and H4. Histones are associated with DNA forming highly organised nucleosomal structures. Their centre consists of a H3-H3-H4-H4 tetramer which is flanked on two sites by a H2A-H2B dimer each. The histone core particle is surrounded by two coils of the DNA double helix (146 base pairs in total). The nucleosomes are joined in a row in a string-of-pearls fashion, the DNA (linker DNA) is associated with the histone H1 in the region of the bond.

Nucleosomes: Highly purified mononucleosomes isolated from calf thymus.
Nucleosomes are highly organised functional subunits of chromosomes consisting of histones (types H1, H2A, H2B, H3 and H4) and dsDNA. Their centre consists of a H3-H3-H4-H4 tetramer which is flanked on two sides by a H2A-H2B dimer. The histone core particle is surrounded by two coils of the DNA double helix (146 base pairs in total). The nucleosomes are joined in a row like beads on a string. The DNA (linker DNA) is associated with the histone H1 in the region of the bond. Mononucleosomes were prepared by treatment of chromatin with nuclease S7 resulting in degradation of linker DNA and dissociation of Histone H1. The preparation is free of histone H1, as verified by SDS gel electrophoresis and silver staining.

nRNP/Sm: Native U1-nRNP purified by affinity chromatography from calf thymus.
U1-nRNP contains the RNP specific proteins 70K, A and C as well as the Sm specific proteins B, B‘, D, E, F, G.

Sm: Native Sm antigen purified by affinity chromatography from calf thymus.
The antigens nRNP and Sm belong to a group of small ribonucleoproteins (snRNP, small nuclear ribonucleoproteins) which consist of low molecular weight RNA with a high uridine content (U-RNA) complexed with various proteins (molecular weights 9 to 70 kDa). The RNA component is termed U1 to U6, depending on its behaviour in chromatography. Besides the particular RNA, the particles of U-nRNP contain six different core proteins (B, B', D, E, F, G), U1-nRNP additionally contains particle-specific proteins (70K, A, C). Antibodies to U1-nRNP are directed against one or more of the particle-specific proteins 70K, A or C. In contrast, antibodies to Sm can also react with one or more core proteins. The U-nRNP particles are involved in splicing of the pre-mRNA (pre-messenger RNA) - they split off the non-coding mRNA sequences (introns) and insert the coding mRNA sequences (exons) to recreate the messenger RNA.

SS-A/Ro: Native SS-A/Ro antigen purified by affinity chromatography from calf thymus.
The SS-A/Ro antigen is localised in the cell nucleus and is involved in the processing of mRNA to translationally active molecules. It is a small ribonucleoprotein which consists of one RNA molecule (Y1-, Y2-, Y3-, Y4- or Y5-RNA; 80 to 112 bases) and a 60 kDa protein. A 52 kDa protein (Ro-52) is also associated with the SS-A/Ro complex, but whether this protein is a component of the SS-A/Ro complex is controversially discussed in the literature. Anti-SS-A positive patient samples contain antibodies against the native SS-A (60 kDa protein) and might additionally react with the Ro-52 protein. Antibodies exclusively against Ro-52 are not specific for Sjögren's syndrome or SLE and can be found in a number of different disease conditions.

SS-B: Native SS-B antigen purified by affinity chromatography from calf thymus.
The SS-B antigen is a phosphoprotein with a molecular weigth of 48 kDa. It functions in the cell nucleus as a helper protein for RNA polymerase III.

Scl-70: Native Scl-70 antigen purified by affinity chromatography from calf thymus.
The Scl-70 antigen has been identified as the enzyme DNA Topoisomerase-I. The molecular weight of the native antigen is 100 kDa. Originally, only a metabolic product of molecular weight 70 kDa was found in the western blot. The DNA Topoisomerase-I is situated in the nucleoplasm and, in a particularly high concentration, in the nucleolus. The enzyme participates in the replication and transcription of the DNA double helix.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-ENA SLE Profile 2 ELISA (IgG) is ratio 0.1.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs. The mean coefficients of variation are as follows:

Intra-assay variation, n = 20	
Antigen	**CV (%)**
dsDNA	3.8
histones	4.5
nucleosomes	2.9
nRNP/Sm	3.6
Sm	2.3
SS-A	3.0
SS-B	3.8
Scl-70	4.1

Inter-assay variation, n = 4 x 6	
Antigen	**CV (%)**
dsDNA	5.7
histones	4.8
nucleosomes	6.2
nRNP/Sm	4.3
Sm	3.6
SS-A	3.4
SS-B	5.2
Scl-70	4.6

The reactivity of each antigen of the Anti-ENA SLE Profile 2 ELISA (IgG) is standardised by the human reference sera CDC-ANA #1 to #10 of the “Center for Disease Control” (Atlanta, USA). The reactivity of the CDC sera in the EUROIMMUN Anti-ENA SLE Profile 2 ELISA (IgG) is summarised in the following table:

Antigen	CDC-1 homoge-nous/rim	CDC-2 speckled/ SS-B	CDC-3 speckled	CDC-4 RNP	CDC-5 Sm	CDC-6 nucleolar	CDC-7 SS-A	CDC-8 contro-mere	CDC-9 Scl-70	CDC-10 Jo-1
dsDNA	**+ (4.3)**	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
histones	**+ (2.6)**	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
nucleos.	**+ (8.9)**	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
nRNP/Sm	**+ (1.1)**	neg.	**+ (7.4)**	**+ (6.0)**	**+ (11.0)**	neg.	neg.	neg.	neg.	neg.
Sm	**+ (1.1)**	neg.	**+ (1.1)**	neg.	**+ (8.8)**	neg.	neg.	neg.	neg.	neg.
SS-A	neg.	**+ (4.3)**	**+ (3.5)**	neg.	neg	neg.	**+ (4.9)**	neg.	neg.	neg.
SS-B	neg.	**+ (4.9)**	**+ (1.7)**	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Scl-70	neg.	neg.	neg.	neg.	neg	neg.	neg.	neg.	**+ (3.0)**	neg.

neg. (negative): Sample extinction value <1 x cut-off
+ (positive): Sample extinction value ≥1 x cut-off (the value in parentheses indicates the multiple of the cut-off)

The specificity of these sera was determined of the “Center for Disease Control” by immunofluorescence patterns (substrate: HEp-2 cells and primate liver), the results of double immunodiffusion or counter immunoelectrophoresis (the sera are not in any case monospecific).

Reference range: The levels of the anti-ENA antibodies (IgG) were analysed with this EUROIMMUN ELISA in a collective of healthy blood donors. With a cut-off of ratio 1.0 the following prevalences were obtained:

Antibodies against	Prevalence	n
dsDNA	1.5%	206
histones	0%	206
nucleosomes	0%	204
nRNP/Sm	0%	206
Sm	0%	206
SS-A	0%	206
SS-B	0%	206
Scl-70	0.5%	206

Clinical significance

Antibodies (AAb) against nuclear antigens (ANA) are directed against various cell nuclear components. Among the most important nuclear antigens, including cytoplasmic antigens, are nRNP/Sm, Sm, SS-A (Ro), SS-B (La), Scl-70, PM-Scl, Jo-1, centromeres, PCNA, dsDNA, nucleosomes, histones and ribosomal P-proteins. They are mainly components of functional nuclear particles, are bound to nucleic acids or fulfil functions in the cell cycle, e.g. in transcription or translation.

The investigation of ANA and subsequent differentiation within the ANA (or ENA) spectrum contributes greatly to establishing a diagnosis, particularly in the following rheumatic diseases:
- systemic lupus erythematosus (SLE),
- Sharp syndrome (mixed connective tissue disease = MCTD),
- Sjögren's syndrome (SS),
- systemic sclerosis (SSc), and
- poly-/dermatomyositis (PM/DM).

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease which occurs in phases and mainly affects the connective tissue and various organic systems. Worldwide, women are ten times more frequently affected by collagenosis than men, whereby there are regional differences, e.g. 12.5 in 100,000 women in central Europe and up to 100 in 100,000 women in the US have SLE. The predilection age is between 15 and 30 years. The clinical symptoms vary greatly and can include butterfly erythema, discoid hyperkeratotic skin changes, purpura, arthralgia, myalgia, kidney insufficiency, neuropsychiatric abnormalities, polyneuropathy, pericarditis, cardiomyopathy, pleuritis, lung fibrosis, anaemia, hepatomegaly and splenomegaly. An SLE attack is often accompanied by fever.

Drug-induced lupus erythematosus (DILE, drug-induced SLE) can manifest itself as SLE (mostly without CNS or kidney involvement), subacute cutaneous lupus erythematodes (SCLE) or chronic cutaneous lupus erythematosus (CCLE). Frequent symptoms are polyarthralgia, pleuritis and pericarditis. DILE can be caused by procainamide, hydralazine, isoniazid or around 80 other drugs.

Sharp syndrome (mixed connective tissue disease, MCTD) is a multi-symptomatic and multiform mixed connective tissue disease combining clinical symptoms of rheumatoid arthritis, SLE, systemic sclerosis, CREST syndrome (calcinosis cutis, Raynaud's phenomenon, oesophagus motility disorders, sclerodactyly, teleangiectasia) and vasculitides.

Sjögren's syndrome (SS) is a chronic inflammatory autoimmune disease of the exocrine glands which can be found in one to four million people in the US alone. Nine out of ten patients are women. The main clinical feature of primary SS is ocular and oral dryness as a result of the destruction of lachrymal and salivary glands by lymphocytic infiltration. The pancreatic glands, mucous secreting glands of the intestine, bronchia, vagina and sudoriferous glands may also be affected. Around 5% of SS patients develop malignant lymphoma. In secondary SS, primary SS symptoms accompany rheumatoid arthritis (RA), SSc, SLE, PM/DM, primary biliary cirrhosis and autoimmune hepatitis.

Systemic sclerosis (SSc) is a chronic inflammatory autoimmune disease which occurs in phases and is characterised by accumulation of collagen in the skin and inner organs. Main symptoms of SSc include skin thickening and episodes of disturbed blood flow in the fingers (Raynaud's syndrome), particularly in cold weather or if the patient suffers from stress. SSc is further characterised by arthritic joint pains and symptoms in the gastrointestinal tract, lungs, heart, kidneys and other inner organs. SSc is divided into the diffuse form (DSSc), the limited form (LSSc) and PM/SSc or PM/SLE/SSc overlap syndrome. DSSc affects the connective tissue of the lungs, kidneys, oesophagus and heart, with lung sclerosis being the most frequent cause of death. LSSc, which is equated to a large extent with CREST syndrome (calcinosis cutis, Raynaud's phenomenon, oesophagus motility disorder, sclerodactyly, teleangiectasis), affects the extremities rather than the inner organs. PM/SSc overlap syndrome is characterised by myositis, interstitial lung disease, arthritis, Raynaud's phenomenon, fever and hyperkeratosis of the hands.

Autoantibodies (AAb) against DNA consist of two different types of antibody. 1. AAb against double-stranded, native DNA (**anti-dsDNA**). These react mainly with epitopes in the deoxyribose phosphate backbone of the double helix. 2. AAb against single-stranded, denatured DNA (**anti-ssDNA**). These bind mainly to epitopes of purine and pyrimidine bases, but they may also react with epitopes of the deoxyribose phosphate backbone.

Anti-dsDNA occur only in SLE. The prevalence is 60 to 90%. Because of their high specificity, the presence of anti-dsDNA is one of the most important criteria for the diagnosis of SLE.

Anti-histones are AAb against nuclear proteins of the five types H1, H2A, H2B, H3, H4, which together with dsDNA form the nucleosomes. They can be considered as a marker for DILE with a prevalence of 95% to 100% (along with ANA with approx. 95%), particularly in those patients, around 30%, who do not exhibit anti-Sm, anti-nucleosomes and anti-dsDNA.

Around 50 to 75% of patients treated with procainamide and 25 to 30% of those treated with hydralazine develop AAb against histones without or with slight symptoms of SLE during long-term therapy. ANA and anti-histone antibodies persist for years after the drugs have been discontinued and the symptoms have abated.

AAb against histones can also be exhibited by patients with autoimmune diseases other than DILE, such as SLE (prevalence 50 to 70%), SSc, rheumatoid arthritis (RA) with vasculitis (prevalence around 75%) or autoimmune liver diseases.

Anti-nucleosome antibodies (ANuA) are directed against functional subunits of chromosomes, which consist of histones and dsDNA. ANuA are a characteristic serological marker for SLE and can be detected with a specificity of almost 100% using a newly developed, highly purified nucleosome preparation. Their prevalence is 55 to 75%, and almost 100% in severe cases. With this test, no reactions have been found with sera from blood donors or from scleroderma, Sjögren's syndrome or polymyositis patients. The ANuA concentration in the serum is a meaningful parameter for assessing SLE activity. In the exclusively cutaneous form of the disease the ANuA titer can be negative.

As can be seen from the 11 clinical and serological ACR criteria (American College of Rheumatology), the collagenosis SLE presents with varying clinical symptoms. It must be taken into consideration that further serologically detectable autoantibodies aside from ANuA can be responsible for the individual disease picture. Therefore, autoantibodies against dsDNA, Sm, nRNP/Sm, SS-A (Ro), SS-B (La), ribosomal P-proteins and other nuclear antigens should also be investigated.

High **anti-nRNP/Sm** titers are characteristic for Sharp syndrome, whereby the titer correlates with the disease activity. Anti-nRNP/Sm antibodies are also detected in patients with SLE, SSc and PM/DM.

AAb against Sm can be considered as pathognomonic for SLE, along with AAb against dsDNA, nucleosomes and ribosomal P-proteins. Sm AAb are detected in 5 to 40% of SLE patients. Whereas the prevalence in caucasians is approx. 10%, it is much higher in other ethnic groups, e.g. of Arabic, Chinese or Black African background. In American studies investigating a high proportion of non-Caucasians, prevalences of 20 to 40% were found.

Anti-SS-A are detected in 40 to 95% of SS cases. They mostly occur in parallel with autoantibodies against SS-B (anti-La). Autoantibodies against SS-A are also found in 20 to 60% of SLE patients and in neonatal lupus erythematosus (neonatal LE syndrome) with a prevalence of 100%. The antibodies are transmitted diaplacentally to the foetus and often cause congenital AV block in addition to inflammatory reactions when the mother is anti-SS-A or anti-SS-B positive (level I-III).

Note: Differentiation of anti-SS-A antibodies from those against the so-called Ro52 antigen (52 kDa protein, RING dependent E3 ligase) is of decisive diagnostic importance, since antibodies against Ro52 are not disease-specific, but are also detected in myositis, systemic sclerosis, neonatal lupus erythematosus and other collagenoses, primary biliary cirrhosis, autoimmune hepatitis and viral hepatitis.

Antibodies against SS-B are detected in 40 to 95% of SS cases. They mostly occur in parallel with autoantibodies against SS-A (anti-Ro). Autoantibodies against SS-B are also found in 5 to 35% of SLE patients and in neonatal lupus erythematosus (neonatal LE syndrome) with a prevalence of 75 to 80%. The antibodies are transmitted diaplacentally to the foetus and often cause congenital AV block in addition to inflammatory reactions when the mother is anti-SS-A or anti-SS-B positive (level I-III).

AAb against Scl-70 are a marker for systemic sclerosis (SSc) and can be found in 25 to 75% of patients. The prevalence in Japan is lower. The serological detection of anti-Scl-70 is mainly associated with a severe diffuse disease course and poor prognosis (in 25% to 75% of SSc cases), less frequently with limited SSc forms (5 to 30%) and SSc/SLE/PM or SSc/PM overlap syndrome (13%). The pathogenetic connection between SSc and autoantibodies against anti-Scl-70 is not yet fully understood since silicosis patients can also develop these antibodies without having SSc.

Antibodies against	Disease	Prevalence
dsDNA	Systemic lupus erythematosus (SLE)	20% - 90%
Histones	Drug-induced lupus erythematosus (DILE) Systemic lupus erythematosus (SLE) Rheumatoid arthritis (RA) with vasculitis	95% - 100% 50% - 70% approx. 75%
Nucleosomes	Systemic lupus erythematosus (SLE)	55% - 75%
nRNP/Sm	Sharp syndrome (MCTD) Systemic lupus erythematosus (SLE) Systemic sclerosis (SSc) Polymyositis/dermatomyositis (PM/DM) Overlapping polymyositis/SSc	95% - 100% 3% - 47% 2% - 14% 12% - 16% approx. 24%
Sm	Systemic lupus erythematosus (SLE)	5% - 40%
SS-A (Ro)	Sjögren's syndrome (SS) Systemic lupus erythematosus (SLE) Neonatal lupus erythematosus	40% - 95% 20% - 60% 95% - 100%
SS-B (La)	Sjögren's syndrome (SS) Systemic lupus erythematosus (SLE) Neonatal lupus erythematosus	40% - 95% 5% - 35% 75% - 80%
Scl-70	Systemic sclerosis (Ssc) - diffuse form (DSSc) - limited form (LSSc)	25% - 75% 25% - 75% 5% - 30%

Literature references

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