研究用試薬

ANA Screen ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1590-9601-8 G	dsDNA, histones, ribosomal P-proteins, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, centromeres	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative in vitro assay for human autoantibodies of the immunoglobulin class IgG against 10 different antigens: **double-stranded DNA (dsDNA)**, **histones, ribosomal P-proteins, nRNP, Sm, SS-A, SS-B, Scl-70, Jo-1 and centromeres** in serum or plasma for the diagnosis of Sharp syndrome (MCTD), systemic lupus erythematosus, Sjögren's syndrome, progressive systemic sclerosis and poly-/dermatomyositis.

Application: The ANA Screen ELISA provides simultaneous determination of antibodies against 10 different nuclear and cytoplasmic antigens with optional, fully automated processing and objective evaluation of the test results. These antibodies are linked to rheumatic diseases.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with a pool of these ten antigens. In the first reaction step, diluted patient samples are incubated in 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 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
6. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	
LOT Lot description IVD In vitro diagnostic medical device	CE		Storage temperature 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 controls:** 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 controls 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.
For example: dilute 5 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

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

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, controls 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) remaining 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.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I and the Analyzer I-2P and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the **semiquantitative determination** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) controls and the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative control serum serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of healthy subjects (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative. Besides this qualitative interpretation a semiquantitative evaluation of the result is possible by calculating a ratio according to the following formula:

$$\frac{\textbf{Extinction of control or patient samples}}{\textbf{Extinction of the calibrator (cut - off)}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0: negative
Ratio ≥1.0: positive

In the case of positive results in the **EUROIMMUN ANA Screen ELISA (IgG)** we recommend differentiation of the antibodies with the **monospecific EUROIMMUN ELISA**.

For duplicate determination the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

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

Test characteristics

Calibration: For every group of tests performed, the extinction values of the calibrator and the ratio values determined for the positive and negative controls 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 controls 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 coated with an antigen pool consisting of the following antigens:

dsDNA: Highly purified native, double-stranded DNA isolated from salmon testes.

Histones: A mixture of individually purified histone types H1, H2A, H2B, H3 and H4 isolated from bovine thymus.

Ribosomal P-proteins: Ribosomal P-proteins purified by affinity chromatography from calf thymus.
The ribosomal P antigen consists of 3 proteins of the 60S ribosomal subunit. These proteins are designated P0 (molecular weight 38 kDa), P1 (19 kDa) and P2 (17 kDa). The major immunoreactive epitope is localised to the carboxy terminus of all 3 proteins and consists of an identical sequence of 17 amino acids.

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 ribo-nucleoproteins) 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 antigen is localised in the cell nucleus and is involved in the processing of mRNA to translationally active molecules. It is a small ribonucleoprotein consisting of an RNA molecule (Y1, Y2, Y3, Y4 or Y5 RNA: 80 to 112 bases in length) and two different proteins, which are the targets for antibodies against SS-A. Initially, in 1984, a protein of 60 kDa was described as a component of ribonucleoproteins. In 1988, antibodies against a further protein with a molecular weight of 52 kDa (Ro- 52) were detected in anti-SS-A-positive sera by means of Westernblot. However, Ro-52 does not appear to be a stable component of the native ribonucleoprotein particle. Only test systems which use native SS-A 60 kDa as the antigen should be used for the detection of autoantibodies against SS-A in SLE or Sjögren's syndrome (SS). Test systems which include Ro-52 in the antigen substrate are not recommended, since antibodies against Ro-52 are frequently also found in myositis patients. Thus, the specificity of these test systems for SLE and SS is reduced. All anti-SS-A positive sera from SLE or SS patients can be identified using native SS-A 60 kDa as the antigen.

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.

Jo-1: Native Jo-1 antigen (histidyl-tRNA synthetase) purified by affinity chromatography from calf thymus
The Jo-1 antigen is identical to histidyl-tRNA synthetase, a cytoplasmic phosphoprotein with a molecular weight of 50 kDa. It joins the amino acid histidine in the cytoplasm to its corresponding tRNA.

Centromeres: Recombinant centromere protein B. The corresponding human cDNA was expressed with a baculovirus vector in insect cells.
Four different proteins were identified as centromere autoantigens: centromere protein-A (17 kDa), centromere protein-B (80 kDa), centromere protein -C (140 kDa) and centromere protein -D (50 kDa). All sera containing anti-centromere antibodies pre-characterized in indirect immunofluorescence tests are at least reactive with centromere protein-B.

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 ANA Screen ELISA (IgG) is ratio 0.08.

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 test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (ratio)**	**CV (%)**
1	4.9	1.7
2	3.1	1.3
3	1.8	1.1

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (ratio)**	**CV (%)**
1	4.8	3.2
2	3.1	4.3
3	1.8	3.7

Reference range: The level of ANA (IgG) were analysed with this EUROIMMUN ELISA in a panel of 216 healthy blood donors. With a cut-off of ratio 1.0, 0.5% of the blood donors were ANA (IgG) positive.

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 ribo-somal 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.

Polymyositis and **dermatomyositis** are idiopathic myositides (autoimmune myositides) and have an incidence of 1:100,000 per year. Women are affected twice as often as men. A triad of components is discussed as cause of these diseases: genetic (HLA-B8, DRW 52, DRW 53), external (bacteria or viruses, such as Toxoplasma gondii or Coxsackie A virus, “environmental pollutants”) and mental (stress). Dermatomyositis (DM) can occur at any age, whereas polymyositis (PM) mostly manifests itself after the second decade of life and inclusion body myositis (IBM) develops in individuals in their fifties and above.

The main symptoms of PM and DM are muscle weakness and in the advanced stage muscle atrophy. At the beginning of the disease mainly the muscles of the larynx are affected, resulting in a raspy voice, dysphagia and dyspnoea. DM is characterised by livid erythema, particularly periorbital, presternal, and on the knees and elbows, painful capillary lesions in the nail fold and bed, and hyperkeratosis of the hands with fissures. 40% to 70% of affected children and 20% of adults also develop calcinosis of the subcutaneous tissue and muscles. PM is divided into the following forms: primary idiopathic myositis (PM and DM each in 33% of cases), paraneoplastic PM/DM (8 to 20%, not in children), infantile DM with concomitant vasculitis (5 to 10%) and PM/DM overlap syndrome in collagenosis (20%). Paraneoplastic PM/DM is associated with carcinoma/tumours of the stomach, intestine, pharynx, lung, mamma or ovary. In most cases the condition of the patient improves after removal of the tumour.

Electromyogram, muscle and skin biopsy, muscle enzyme titer determination and specific autoimmune serology contribute to establishing a diagnosis. The investigation of PM/DM-associated autoantibodies using special tests is indispensable for the diagnosis of PM/DM and the assessment of the disease course and therapy success.

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-ribosomal P-protein autoantibodies (ARPA) are directed against specific ribosomal phosphoproteins. ARPA are considered a highly specific marker for the diagnosis of SLE. They are very rarely found in other autoimmune diseases, e.g. in SLE/MCTD overlap syndrome (SLE/Sharp syndrome). The prevalence of ARPA in SLE patients is between 5% and 46%, with Asian patients ranging at the upper end and Black Africans and Caucasians at the lower end. The investigation of ARPA is indicated in suspected cases of SLE and lupus-induced psychosis. A connection between the ARPA titer level and SLE activity is being controversially discussed.

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.

Anti-Jo-1 are autoantibodies against histidyl-tRNA synthetase ($tRNA^{his}$ synthetase). Antibodies against Jo-1 are an acknowledged and highly specific marker for PM/DM. There prevalence in PM/DM is 18 to 30% (with a PM/DM ratio of 2:1). 60% of patients who are positive for anti-Jo-1 antibodies develop the so-called anti-synthetase syndrome, which is characterised by a complex of symptoms: myositis, interstitial lung disease, arthritis, Raynaud's phenomenon, fever and hyperkeratosis of the hands.

Anti-centromere antibodies (ACA) are directed against centromere proteins. The serological detection of ACA is relevant for both diagnostics and differentiation. ACA can be found in 20 to 30% of SSc patients, most frequently in Caucasians. In most cases, ACA are associated with LSSc. The presence of ACA, with a prevalence of 80 to 90%, is considered an indicator of a mild disease course and good prognosis. In DSSc, which also includes lung fibrosis, ACA are detected in around 8% of patients. Furthermore, 15 to 30% of patients with primary biliary cirrhosis (PBC), which is also an autoimmune disease, express ACA.

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%
Ribosomal P-proteins	Systemic lupus erythematosus (SLE)	5% - 46%
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%
Jo-1	Polymyositis/dermatomyositis (PM/DM)	18% - 30%
Centromeres	Systemic sclerosis (Ssc) - limited form (LSSc) - diffuse form (DSSc) Primary biliary cirrhosis (PBC)	20% - 30% 80% - 95% approx. 8% 15% - 30%

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