# EUROLINE ANA Profile 23 (IgG)
# Test instruction

| ORDER NO. | ANTIBODIES AGAINST | IG CLASS | SUBSTRATE | FORMAT |
|---|---|---|---|---|
| DL 1590-1601-23 G<br>DL 1590-6401-23 G | dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, nRNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP A, CENP B, Sp100, PML, Scl-70, PM-Scl100, PM-Scl75, RP11, RP155, gp210, PCNA, DFS70 | IgG | Ag-coated immunoblot strips | 16 x 01 (16)<br>64 x 01 (64) |

**Indications:** The EUROLINE test kit provides qualitative in vitro determination of human autoantibodies of the immunoglobulin class IgG to the 23 different antigens: **dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, nRNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP A, CENP B, Sp100, PML, Scl-70, PM-Scl100, PM-Scl75, RP11, RP155, gp210, PCNA and DFS70** in serum or plasma to support the diagnosis of Sharp syndrome (MCTD), systemic lupus erythematosus (SLE), Sjögren's syndrome, progressive systemic sclerosis, poly-/dermatomyositis, overlap syndrome, limited form of progressive systemic sclerosis (CREST syndrome) and primary biliary liver cholangitis.

**Application:** In an international consensus (www.anapatterns.org, 2014) for the standardised nomenclature of HEp-2 cell patterns in indirect immunofluorescence, different cell nucleus patterns were defined (pattern, AC1–XX). The EUROLINE ANA-Profile 23 provides a multiplex approach for the confirmation and differentiation of 23 autoantibodies which cause the nuclear patterns described in the consensus, in only one incubation, with optional fully automated processing and objective evaluation of test results using the EUROLineScan software.

**Principles of the test:** The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. 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 | Format | Format | Symbol |
|---|---|---|---|
| **1. Test strips coated with the antigens** dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, nRNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP A, CENP B, Sp100, PML, Scl-70, PM-Scl100, PM-Scl75, RP11, RP155, gp210, PCNA and DFS70 | 16 strips | 4 x 16 strips | STRIPS |
| **2. Positive control** (IgG, human), 100x concentrate | 1 x 0.02 ml | 4 x 0.02 ml | POS CONTROL 100x |
| **3. Enzyme conjugate** Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate | 1 x 3 ml | 4 x 3 ml | CONJUGATE 10x |
| **4. Sample buffer**, ready for use | 1 x 100 ml | 3 x 100 ml | SAMPLE BUFFER |
| **5. Wash buffer**, 10x concentrate | 1 x 50 ml | 1 x 100 ml | WASH BUFFER 10x |
| **6. Substrate solution** Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use | 1 x 30 ml | 4 x 30 ml | SUBSTRATE |
| **7. Incubation tray** | 2 x 8 channels | --- | |
| **8. Test instruction** | 1 booklet | 1 booklet | |

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

**Modifications to the former version are marked in grey.**

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips

## Preparation and stability of the reagents

**Note:** This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips the package should be sealed tightly and stored at +2°C to +8°C.

- **Positive control:** The control is a 100x concentrate. For the preparation of the ready for use control the amount required should be removed from the bottle using a clean pipette tip and diluted 1:101 with sample buffer. Example: add 15 µl of control to 1.5 ml of sample buffer and mix thoroughly. The ready for use diluted control should be used at the same working day.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with sample buffer. For one test strip, dilute 0.15 ml enzyme conjugate with 1.35 ml sample buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

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

- **Wash buffer:** The wash buffer is supplied as a 10x concentrate. For the preparation of the ready for use wash buffer the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with distilled water. For one test strip, dilute 1 ml in 9 ml of distilled water. The ready for use diluted wash buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

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

**Waste disposal:** Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

**Warning:** The control of human origin has 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 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:** The **patient samples** for analysis are diluted **1:101** with sample buffer using a clean pipette tip. For example, add 15 µl of sample to 1.5 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

## Incubation

| | |
|---|---|
| **Pretreat:** | Remove the required amount of test strips from the package and place them each in an empty channel (Make sure that the surface of the test trips is not damaged!) The number on the test strip should be visible. Fill the channels of the incubation tray according to the number of serum samples that should be tested with 1.5 ml sample buffer each.<br>Incubate for **5 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid. |
| **Incubate:** (1st step) | Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip.<br>Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. |
| **Wash:** | Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength wash buffer on a rocking shaker. |
| **Incubate:** (2nd step) | Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-labelled anti-human IgG) into each channel.<br>Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. |
| **Wash:** | Aspirate off the liquid from each channel. Wash as described above. |
| **Incubate:** (3rd step) | Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. |
| **Stop:** | Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with distilled water. |
| **Evaluate:** | Place test strip on the evaluation protocol, air dry and evaluate. |

For automated incubation with the **EUROBlotMaster** select the program **Euro01 AAb EL30**.

For automated incubation with the **EUROBlotOne** select the program **EURO 01/02**.

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## Incubation protocol

**Pretreat**
Put the test strip into the incubation channel and fill each channel with 1.5 ml sample buffer

5 min — Shake

**1. Step: Incubate**
Aspirate off, pipette 1.5 ml of diluted serum sample (1:101) into the incubation channel

30 min — Shake

**Wash**
Aspirate off, wash 3 x 5 min with 1.5 ml working-strength wash buffer each

**2. Step: Incubate**
Aspirate off, pipette 1.5 ml enzyme conjugate into the incubation channel

30 min — Shake

**Wash**
Aspirate off, wash 3 x 5 min with 1.5 ml working-strength wash buffer each

**3. Step: Incubate**
Aspirate off, pipette 1.5 ml substrate into the incubation channel

10 min — Shake

**Stop**
Aspirate off, rinse three times with 1.5 ml distilled water

**Evaluation**
EUROLineScan (digital)

## Interpretation of results

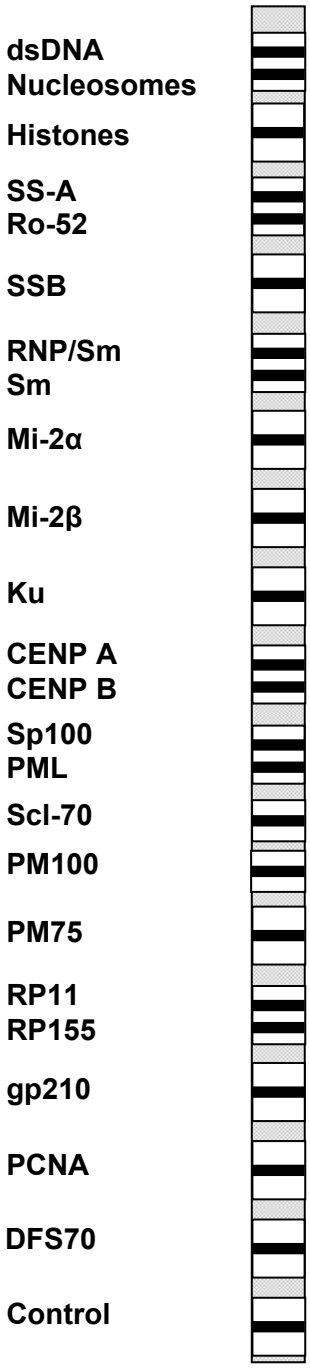
**Handling:** For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (YG_0006_A_UK_CXX, EUROIMMUN). The code for entering the **test** in the EUROLineScan is **ANA_23**.

**Note:** Correct performance of the incubation is indicated by an intense staining of the control band. A white band at the position of an antigen has to be interpreted as negative.

**Antigens, associated HEp2 nuclear patterns (Anti-Cell pattern, AC1 - XX) and their arrangement on the strips:** The EUROLINE test strips have been coated with the following antigens:

| Antigen | AC | Description |
|---|---|---|
| **dsDNA** | AC-1 | Highly purified, native double stranded DNA |
| **Nucleo-somes** | AC-1 | Native higly purified mononucleosomes |
| **Histones** | AC-1 | Highly purified histones |
| **SS-A** | AC-4 | Highly purified SS-A-antigen (60 kDa, native) |
| **Ro-52** | -* | Recombinant Ro-52 (52 kDa) |
| **SS-B** | AC-4 | Highly purified SS-B-antigen (native) |
| **nRNP/Sm** | AC-5 | Highly purified U1-nRNP (native) |
| **Sm** | AC-5 | Highly purified Sm-antigen (native), the main component of the Sm-preparation is presented by the D protein |
| **Mi-2α** | AC-4 | Recombinant Mi-2α protein |
| **Mi-2β** | AC-4 | Recombinant Mi-2β protein |
| **Ku** | AC-4 | Recombinant Ku protein |
| **CENP A** | AC-3 | Recombinant centromere protein A |
| **CENP B** | AC-3 | Recombinant centromere protein B |
| **Sp100** | AC-6 | Recombinant Sp100 protein |
| **PML** | AC-6 | Recombinant PML protein |
| **Scl-70** | AC-29 | Highly purified Scl-70 antigen (DNA topoisomerase I) |
| **PM-Scl100** | AC-8 | Recombinant PM-Scl100 protein |
| **PM-Scl75** | AC-8 | Recombinant PM-Scl75 protein (75 kDa) |
| **RP11** | AC-10* | Recombinant subunit POLR3K of human RNA polymerase III |
| **RP155** | AC-10* | Recombinant subunit POLR3A of human RNA polymerase III |
| **gp210** | AC-11* | Recombinant gp210 protein |
| **PCNA** | AC-13 | Recombinant PCNA (36 kDa) |
| **DFS70** | AC-2 | Recombinant DFS70 (full length) |

* The allocation of antigens to the AC classifications was made based on [4] and the known patterns of EUROIMMUN HEp-2 cells. Ro-52 was not allocated to any AC. RP11 and RP155 present a pattern on the EUROIMMUN HEp-2 cells which corresponds to AC-10, in [4], RNA polymerase AC-5 is allocated. On the EUROIMMUN HEp-2 cells, gp210 shows an AC-11, in [4], gp210 AC-12 is allocated.



EUROIMMUN recommends interpreting results based on the signal intensity:

| Signal intensity EUROLineScan Flatbed scanner | Result | |
|---|---|---|
| 0-5 | o | Negative |
| 6-10 | (+) | Borderline |
| 11-25 or 26-50 | +, ++ | Positive |
| >50 | +++ | Strong positive |

Results in the **borderline range** (+) should be evaluated as increased but negative. The table above contains **values** for the evaluation using a flatbed scanner. The **values** for other instruments supported by EUROLineScan can be found in the EUROLineScan program. To do so mark the corresponding assay in the test list (main menu "Help" → "Test") and click on details and select **the corresponding instrument** in **"image source"**.

An indirect immunofluorescence test should always be performed in parallel with the determination of cell nucleus antibodies by EUROLINE. On the one hand, this provides a check on plausibility as a safeguard against false-positive 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 EUROLINE.

For the medical diagnosis, the clinical symptoms of the patient and, if available, further findings should always be taken into account alongside the serological result. A negative serological result does not exclude the presence of a disease.

## Test characteristics

**Calibration:** The reactivity of each antigen is standardised by the human reference sera CDC-ANA #1 to #11 of the "Centers for Disease Control and Prevention" (CDC, Atlanta, USA). The reactivity of the CDC sera in the EUROIMMUN EUROLINE is summarised in the following table:

| Antigen | CDC-1 Homogeneous/ rim | CDC-2 Speckled/ SS-B | CDC-3 Speckled | CDC-4 RNP | CDC-5 Sm | CDC-6 Nucleolar | CDC-7 SS-A | CDC-8 Centromere | CDC-9 Scl-70 | CDC-10 Jo-1 | CDC-11 PM-Scl |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **nRNP/Sm** | **pos.** | neg. | **pos.** | **pos.** | **pos.** | neg. | neg. | neg. | neg. | neg. | neg. |
| **Sm** | **pos.** | neg. | **pos.** | neg. | **pos.** | neg. | neg. | neg. | neg. | neg. | neg. |
| **SS-A** | neg. | **pos.** | **pos.** | neg. | neg. | neg. | **pos.** | neg. | neg. | neg. | neg. |
| **Ro-52** | neg. | **pos.** | **pos.** | neg. | neg. | neg. | **pos.** | neg. | neg. | **pos.** | neg. |
| **SS-B** | neg. | **pos.** | **pos.** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |
| **Scl-70** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. | neg. |
| **PM-Scl** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | **pos.** |
| **Jo-1** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. |
| **CENP B** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. | neg. | neg. |
| **PCNA** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |
| **dsDNA** | **pos.** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |
| **Nucleosomes** | **pos.** | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. | neg. | neg. | neg. |
| **Histones** | **pos.** | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. | neg. | neg. | neg. |
| **Rib. P protein** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |
| **AMA-M2** | neg. | neg. | neg. | neg. | neg. | neg. | neg. | **pos.** | neg. | neg. | neg. |

The specificity of these sera was determined at the CDC 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).

**Measurement range:** The EUROLINE is a qualitative method. No measurement range is provided.

**Cross reactions:** The high analytical specificity of the test system is guaranteed by the quality of the antigen substrates used (antigens and antigen sources). This EUROLINE specifically detects IgG class antibodies to dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, nRNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP A, CENP B, Sp100, PML, Scl-70, PM-Scl100, PM-Scl75, RP11, RP155, gp210, PCNA and DFS70. No cross reactions with other autoantibodies have been found.

**Interference:** Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml haemoglobin, of 20 mg/ml triglycerides and of 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

**Inter- and intra-assay variation:** The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

**Sensitivity and specificity:**
**dsDNA:** For the detection of autoantibodies against dsDNA a sensitivity of 94% with reference to the ELISA method was determined using 36 samples of patients with SLE. The specificity was 100% for healthy blood donors (n = 50) and for a panel of non-SLE rheumatic diseases (Sjögren's syndrome n = 14, systemic sclerosis n = 18).

**Nucleosomes:** For the detection of autoantibodies against nucleosomes a sensitivity of 97% with reference to the EUROIMMUN Anti-Nucleosomes ELISA (IgG) method was determined using 34 samples of patients with SLE. The clinical prevalence determined by the ELISA (CE-notified test, coated with native mononucleosomes free from histone H1 and non-histone proteins, Patent EP1476750B1/US7566545 (B2)) amounts to 53%. The specificity was 100% for healthy blood donors (n = 50) and in a panel of non-SLE rheumatic diseases (Sjögren`s syndrome n = 14, systemic sclerosis n = 18).

**Histones:** For the detection of autoantibodies against histones a sensitivity of 78% with reference to the ELISA method was determined using 41 samples of patients with SLE. The specificity was 100% for healthy blood donors (n = 50) and 97% in a panel of non-SLE rheumatic diseases (Sjögren's syndrome n = 14, systemic sclerosis n = 18).

**SS-A:** For the detection of autoantibodies against SS-A a sensitivity of 100% with reference to the ELISA method was determined using 14 samples of patients with Sjögren's syndrome. The specificity was 100% for healthy blood donors (n = 50) and 95% in a panel of non-SLE rheumatic diseases (systemic sclerosis n = 18, MCTD n = 22).

**Ro-52:** For the detection of autoantibodies against Ro-52 a sensitivity of 100% with reference to the westernblot method was determined using 103 samples of patients with SLE and Sjögren's syndrome (SLE n = 23, Sjögren's syndrome n = 77 and neonatal lupus erythematosus n = 3). The specificity was 100% for healthy blood donors (n = 65). Antibodies against Ro-52 are not disease specific and can be detected in samples from patients suffering from myositis, systemic sclerosis and other rheumatic diseases, i.e. in 7 of 20 samples of systemic sclerosis patients autoantibodies against Ro-52 were detected.
**Isolated antibody reactions with Ro-52** should not be evaluated as anti-SS-A positive or specific for SLE or Sjögren's syndrome, since they can occur in many different autoimmune diseases.

We recommend interpreting the EUROLINE with reference to the ANA screening test (HEp-2 cells/primate liver) as follows:

| **IIFT** | **EUROLINE** | | **Result** |
|---|---|---|---|
| **HEp-2 cells** | **Ro-52 (52 kDa)** | **SS-A (60 kDa)** | |
| ANA negative | positive | negative | Anti-SS-A negative |
| ANA positive | positive | negative | Anti-SS-A negative |
| ANA positive | positive or negative | positive | Anti-SS-A positive |

It has been shown in various studies that anti-SS-A positive sera always contain antibodies against native SS-A (60 kDa protein) and may additionally exhibit antibodies against Ro-52. For example, in a Japanese study (EUROIMMUN) sera from 103 patients with SLE and Sjögren's syndrome (SLE n = 26, Sjögren's syndrome n = 77), which were characterised as anti-SS-A positive by double immunodiffusion, were investigated. 102 sera reacted with native SS-A, and 90 sera reacted additionally with the Ro-52 band. But no serum showed only a reaction with the Ro-52 band. This study demonstrates that antibodies against native SS-A can be reliably detected using the native SS-A. In rare cases and in suspected cases of neonatal lupus syndrome, the Ro-52 band may provide important supplementary information.

**SS-B:** For the detection of autoantibodies against SS-B a sensitivity of 100% with reference to the ELISA method was determined using 14 samples of patients with Sjögren's syndrome. The specificity was 100% for healthy blood donors (n = 50) and 97% in a panel of non-SLE rheumatic diseases (systemic sclerosis = 18, MCTD n = 22).

**nRNP/Sm:** For the detection of autoantibodies against RNP/Sm a sensitivity of 100% with reference to the ELISA method was determined using 22 samples of patients with MCTD (mixed connective tissue disease). The specificity was 100% for healthy blood donors (n = 50) and 96% in a panel of non-SLE rheumatic diseases (Sjögren's syndrome n = 14, systemic sclerosis n = 18, polymyositis n = 25).

**Sm:** For the detection of autoantibodies against Sm a sensitivity of 100% with reference to the ELISA method was determined using 45 samples of patients with SLE. The specificity was 100% for healthy blood donors (n = 50) and 100% in a panel of non-SLE rheumatic diseases (Sjögren's syndrome n = 14, systemic sclerosis n = 18, polymyositis n = 25).

**Mi-2α:** Sera from 264 patients with clinically characterised myositis and 120 control samples were investigated for antibodies against Mi-2α. The prevalence amounted to 7%, at a specificity of 100%.

**Mi-2β and Ku:** Sera from 153 patients with clinically characterised myositis (50 patients with dermatomyositis, 89 patients with polymyositis, 4 patients with juvenile dermatomyositis and, 10 patients with inclusion body myositis) and 77 sera from control patients (26 patients with Sjögren syndrome, 26 patients with SLE and 25 patients with systemic sclerosis) were investigated for antibodies against Mi-2β and Ku. There were prevalences of 3% each, at a specificity for myositis of 100% and 97%, respectively.

**Scl-70, CENP A, CENP B, RP11, RP155, PM-Scl100 and PM-Scl75:** 129 sera from patients with clinically characterised systemic sclerosis (SSc, diffuse and limited form) as well as 202 sera from control patients (50 patients with dermato/polymyositis, 50 with systemic lupus erythematosus, 42 with rheumatoid arthritis and 60 healthy blood donors) were tested for antibodies against Scl-70, CENP A, CENP B, RP11 and RP155 (RNA Polymerase III subunits), PM-Scl100 and PM-Scl75. Sensitivities and specificities were calculated by ROC analysis at the given cut-off value of 10 intensity units of the EUROLINEScan program.

| Anti- | Sensitivity [%] | Specificity [%] |
|---|---|---|
| **Scl-70** | 65.1 | 98.5 |
| **CENP A** | 10.9 | 98.5 |
| **CENP B** | 13.2 | 98.5 |
| **RP11** | 5.4 | 99.5 |
| **RP155** | 7.0 | 100.0 |
| **PM-Scl100** | 6.6 | 99.0 |
| **PM-Scl75** | 11.8 | 98.0 |

**Sp100, PML and gp210:** Sera from 170 patients with clinically characterised primary biliary liver cholangitis (PBC), 49 sera from patients with autoimmune hepatitis (AIH), 200 sera from patients with viral hepatitis (HCV or HBV) were investigated for the presence of antibodies against Sp100, PML and gp210. Based on this study, four patients from the AIH patient panel were diagnosed with PBC/AIH overlap syndrome.

| Anti- | Sensitivity [%] | Specificity [%] |
|---|---|---|
| **Sp100** | 20.6 | 99.2 |
| **PML** | 12.9 | 98.8 |
| **gp210** | 26.5 | 98.8 |

**PCNA:** In 13 of 20 patient sera, having a cyclin I-positive pattern in the indirect immunofluorescence (HEp-2-cells/primate liver), autoantibodies against PCNA were detected. The specificity was 100% for healthy blood donors (n = 50) and 99% in cyclin I-negative sera of patients with SLE (n = 83).

**DFS70:** The investigation of sera from 198 healthy blood donors showed a prevalence of 3.5% (n = 7) for autoantibodies against DFS70. All positive samples showed a granular ANA pattern and a granular colouring of the chromosomes (typical of anti-DFS70) in the indirect immunofluorescence test with HEp-2 cells. The investigation of sera from 50 samples with positive, partly unclear ANA pattern showed, with respect to the reference method ELISA, a sensitivity of 92.3% at a specificity of 91.7% for the detection of autoantibodies against DFS70. With respect to the reference method Westernblot (whole cell lysate from HEp-2 cells with specific detection of the DFS70 band), the investigation showed a sensitivity of 100% at a specificity of 85.7% in the same samples (n = 49).

**Reference range:** The reference range was determined by incubating samples from healthy blood donors (n = 50). All blood donors were negative (exception see DFS70).

## Clinical significance

Antibodies against nuclear antigens (ANA) are directed against various cell nuclear components (biochemical substances in the cell nucleus). These encompass nucleic acids, cell nuclear proteins and ribonucleoproteins. The serological detection of autoantibodies against individual or several cell nuclear autoantigens is an essential element in the diagnosis of autoimmune diseases, particularly rheumatic diseases. The frequency (prevalence) of anti-nuclear antibodies in inflammatory rheumatic diseases is between 20% and 100% (in rheumatoid arthritis between 20% and 40%). Therefore, differential ANA diagnostics to detect autoantibodies against different nuclear antigens is indispensable for the identification of individual rheumatic diseases. ANA analysis is also helpful in the diagnosis of other autoimmune diseases, such as primary biliary cholangitis (PBC) or autoimmune hepatitis (AIH).

The ANA profiles offer innovative test combinations based on the lineblot technology (EUROLINE). Positive test results provide important serodiagnostic information for the diagnosis of the rheumatic diseases below, as well as further autoimmune diseases such as PBC.

1. Systemic lupus erythematosus (SLE)

   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.

   In drug-induced lupus around 50 to 75% of patients treated with procainamide and 25 to 30% of those treated with hydralazine develop ANA without symptoms of SLE during long-term therapy. A third of these patients demonstrate autoantibodies against histones and after varied duration of therapy show polyarthalgia, pleuritis and pericarditis. These ANA persist for years after the drugs have been discontinued and the symptoms have abated.

2. Sharp syndrome (mixed connective tissue disease = MCTD)

   Sharp syndrome is a multi-symptomatic and multiform MCTD combining symptoms of rheumatoid arthritis (RA), SLE, systemic sclerosis (SSc) and polymyositis. It has not yet been clarified if it is an independent disease.

3. Sjögren's syndrome (primary Sjögren's syndrome, SS)

   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, the mucous secreting glands of the intestine, bronchia or vagina and the sudoriferous glands may also be affected. Around 5% of SS patients develop malignant lymphoma. In secondary SS the disease signs of primary SS occur as accompanying symptoms of RA, SSc, SLE, polymyositis/dermatomyositis, PBC and AIH.

4. Systemic sclerosis (systemic scleroderma, SSc)

   SSc is an autoimmune connective tissue disease, which affects the skin and the inner organs. It affects around 2 to 50 in 100,000 persons worldwide (USA: 25 in 100,000), and is around three to four times more common in women than in men.

   Shortening of the lingual frenum and Raynaud's syndrome are early symptoms of SSc. In the following phase oedema of the hands and feet develop. The skin becomes stiff and in later stages atrophic, waxy and thin. Finally, deformation of the hands occurs. The fingers become fixed in a bent position (claw hand) and are highly tapered at the ends (Madonna fingers). Furthermore, the characteristic masklike face with rigid mimic develops. Finally, callosity of the inner organs, particularly of the digestive tract, lungs, heart and kidneys occurs. At present, lung involvement is the most frequent cause of death from SSc. Manifest SSc is the collagenosis with the highest vital risk for the patient. The 10-year survival rate is 55%.

   SSc is divided into limited and diffuse forms, depending on the cutaneous distribution. In the limited form, skin involvement is limited to the distal extremities. In the diffuse form (also proximal systemic sclerosis) the symptoms are diffusely distributed over the trunk, the proximal and distal extremities and the face.

5. Myositis (poly-/dermatomyositis)

   The autoimmunogenic myositides (idiopathic inflammatory myopathies) are systemic autoimmune diseases with inflammation of the skeletal musculature, symmetric and proximal accentuated pain and muscle weakness. They occur with an incidence of 0.1-1 per 100,000 per year, a prevalence of 1-6 per 100,000 and ratio of men to women of 1 to 2. They can be divided into polymyositis of adults (around 30%), dermatomyositis of adults (around 30%), paraneoplastic polymyositis of the lungs, ovaries, mammary glands, gastrointestinal tract and in myeloproliferative diseases (around 8%), infantile myositis/dermatomyositis with accompanying vasculitis (around 7%), as well as myositides in association with autoimmune diseases such as RA, lupus erythematosus, MCTD and rare forms such as granulomatosis, eosinophile, focal and inclusion body myositis (around 20%). It should be noted that dermato-/polymyositis is often of paraneoplastic origin, particularly in elderly patients. Dermatomyositis symptoms can occur before the tumour is even diagnostically detectable.

   Polymyositis (PM) is a systemic inflammatory disease of the skeletal muscles of unknown aetiology with perivascular lymphocytic infiltration. When the skin is involved, the disease is known as dermatomyositis (DM). Clinical symptoms of PM are recurring bouts of fever, muscle weakness, arthralgia, possibly Raynaud's syndrome, trouble with swallowing and involvement of the inner organs. In DM, skin symptoms appear as purple-coloured exanthema on the eye lids, nose bridge and cheeks, periorbital oedema, local erythema and scaly eczema dermatitis.

6. Rheumatoid arthritis (RA)

   RA is both one of the most common autoimmune disorders and also the most common chronic inflammatory joint disease. The disease affects around 1% of the world population, whereby 75% of patients are female. It is characterised by inflammation of the synovial membrane, which spreads symmetrically from the small to large joints leading to the destruction of the joints in the late phase accompanied by a systemic involvement of the soft tissue. Initial symptoms include painful swelling of basic finger joints with morning stiffness in the joints. Reliable and earliest possible diagnosis is indispensable to keep the disease under control with suitable therapy and to avoid irreversible joint damage.

7. Primary biliary cholangitis (PBC)

   PBC is a chronic non-suppurative destructive cholangitis with progressive inflammatory destruction of the small biliary ducts and liver cirrhosis in the final stage. In 80 to 90% of cases the patients are female, mainly between 20 and 60 years of age. In rare cases, the disease also affects children. In Germany the prevalence is around 3 to 4 cases per 100,000 inhabitants. Demographic differences (Caucasians, Africans, etc.) are minimal.

   PBC can be subdivided into various stages using liver biopsy. In around 6% of cases there is an increased risk of hepatocellular carcinoma. In the final stage of PBC (decompensated cirrhosis) only liver transplantation will save the patient's life. In around 75% of cases the transplant patients recover fully from PBC. Some patients, however, suffer a PBC relapse after transplantation, but only with a very slow disease course.

   In addition to the typical PBC histological characteristics, specific serodiagnostic parameters are important for confirming suspected cases of PBC: 1. Biochemical markers of cholestasis, such as increased levels of alkaline phosphatase (AP) and gamma-glutamyl transferase (γGT) in serum, 2. Presence of PBC-specific autoantibodies, in particular autoantibodies against mitochondria (AMA) which are directed against the component M2 (family of oxo-acid dehydrogenases), and 3. Additional determination of ANA, in particular against nuclear granules (nuclear dots, sp100 and PML) and against nuclear membrane (gp210), which are also pathognomonically relevant. Autoantibodies against centromere proteins are found regularly in a proportion of patients with overlap syndrome with SSc.

Overview

| **Autoantibodies against** | **Autoimmune disease** | **Prevalence** |
|---|---|---|
| nRNP/Sm | MCTD | 95% |
| Sm | SLE | 5% - 40% |
| SS-A | SS or SLE<br>Neonatal lupus erythematosus | 40% - 95% or 20% - 60%<br>95% - 100% |
| Ro-52 | SS or SLE<br>SSc or idiopathic inflammatory myopathy | 70% - 90% or 40% - 60%<br>20% or 20% - 40% |
| SS-B | SS or SLE<br>Neonatal lupus erythematosus | 40% - 95% or 10% - 20%<br>75% |
| Scl-70 | SSc<br>Diffuse or limited form of SSc | 25% - 75%<br>40% - 65% or 5% - 15% |
| PM-Scl | SSc including overlap syndrome<br>PM/SSc overlap syndrome<br>SSc (anti-PM-Scl75 positive)<br>SSc (anti-PM-Scl100 positive) | 10% - 20% or 5% - 20%<br>18%<br>24% - 50%<br>7% |
| Jo-1 | Myositis (polymyositis/dermatomyositis) | 25% - 35% |
| CENP A | SSc - limited form or SSc - diffuse form | 80% - 95% or 5% - 10% |
| CENP B | SSc - limited form or SSc - diffuse form<br>PBC | 80% - 95% or 8%<br>10% - 30% |
| PCNA | SLE | 3% |
| dsDNA | SLE | 40% - 90% |
| Nucleosomes | SLE | 40% - 70% |
| Histones | Drug-induced SLE<br>SLE or RA | 95% - 100%<br>50% or 15% - 50% |

| Autoantibodies against | Autoimmune disease | Prevalence |
|---|---|---|
| ribosomal P-protein | SLE | 10% |
| AMA M2: | PBC or other chronic liver diseases<br>SSc | up to 96% or 30%<br>7% - 25% |
| DFS70 | Atopic dermatitis<br>Rheumatic diseases | 4% - 10%<br>5% - 10% |
| Mi-2α | DM | approx. 20% |
| Mi-2β | DM, associated with neoplasia (e.g. colon or breast carcinoma) | approx. 10% |
| Ku | SLE/myositis/SSc | up to 10%/40%/5% |
| RP11 | SSc | 5% |
| RP155 | SSc | 7% |
| Sp100 | PBC | 21% |
| PML | PBC | 13% |
| gp210 | PBC | 26% |

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