# EUROLINE Systemic sclerosis (Nucleoli) profile (IgG)
# Test instruction

| ORDER NO. | ANTIBODIES AGAINST | IG CLASS | SUBSTRATE | FORMAT |
|---|---|---|---|---|
| DL 1532-1601 G<br>DL 1532-6401 G<br>DL 1532-5001 G | Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, Ro-52 | IgG | Ag-coated immunoblot strips | 16 x 01 (16)<br>64 x 01 (64)<br>50 x 01 (50) |

**Indications:** The EUROLINE test kit provides qualitative in vitro determination of human autoantibodies of the immunoglobulin class IgG to the 13 different antigens **Scl-70, CENP A, CENP B, RP11 and RP155 (RNA Polymerase III subunits), fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR (platelet derived growth factor receptor) and Ro-52** in serum or plasma to support the diagnosis of progressive systemic sclerosis (SSc, diffuse and limited form) and overlap syndromes.

**Application:** The newly developed EUROLINE Systemic sclerosis (Nucleoli) profile (IgG) is the first membrane-based test system to offer such a comprehensive spectrum of systemic sclerosis-associated antigens, thus allowing the differentiation of antibodies that show a nucleolar pattern in indirect immunofluorescence. The test surpasses all existing detection methods with respect to sensitivity and specificity and enables secure and accurate detection of autoantibodies in systemic sclerosis and overlap syndrome. The test completes the comprehensive range of EUROLINE products, which can also be processed using a user-friendly fully automated blot processor and an evaluation 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.
The format DL 1532-5001 G belongs to the Immunoblot-PreQ system. The test strips are already placed into the incubation trays (EUROTray).

**Contents of the test kit:**

| Component | Format | Format | Format | Symbol |
|---|---|---|---|---|
| **1. Test strips coated with the antigens** Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR and Ro-52 | 16 strips | 4 x 16 strips | 5 x 10 strips in EUROTrays | STRIPS |
| **2. Positive control** (IgG, human), 100x concentrate | 1 x 0.02 ml | 4 x 0.02 ml | 5 x 0.1 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. Enzyme conjugate** Alkaline phosphatase-labelled anti-human IgG (goat), ready for use | --- | --- | 4 x 30 ml | CONJUGATE |
| **5. Sample buffer** ready for use | 1 x 100 ml | 3 x 100 ml | 2 x 100 ml | SAMPLE BUFFER |
| **6. Wash buffer** 10x concentrate | 1 x 50 ml | 1 x 100 ml | 1 x 100 ml | WASH BUFFER 10x |
| **7. Substrate solution** Nitrobluetetrazoliumchloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use | 1 x 30 ml | 4 x 30 ml | 4 x 30 ml | SUBSTRATE |
| **8. Incubation tray** | 2 x 8 channels | --- | --- | |
| **9. Test instruction** | 1 booklet | 1 booklet | 1 booklet | |

| | | | |
|---|---|---|---|
| LOT  Lot description | C E | ✗ | 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)

**If using Immunoblot-PreQ (DL 1532-5001 G), no additional incubation tray is needed.**

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

If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under:

ZD 1532-0101 G Visual evaluation protocol EUROLINE Systemic sclerosis (Nucleoli) profile (IgG).

If using Immunoblot-PreQ (DL 1532-5001 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

## 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/Immunoblot-PreQ 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 and diluted 1:10 with sample buffer. For one test strip, dilute 0.15 ml enzyme conjugate with 1.35 ml sample buffer. The diluted enzyme conjugate should be used at the same working day.

- **Enzyme conjugate:** Ready for use
  Note: Only for DL 1532-5001 G!

- **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 to +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

If using Immunoblot-PreQ (DL 1532-5001 G), manual incubation is not possible. Please see below for options of automated 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 strips 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>Use of Immunoblot-PreQ: Set up the required antigen profiles according to the work protocol and insert into the incubation device.<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.<br>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.<br>Immunoblot-PreQ: The evaluation of the test strips is realised exclusively via the EUROIMMUN camera systems. |

For automated incubation with the **EUROBlotMaster** select the program **Euro01 AAb EL30**.
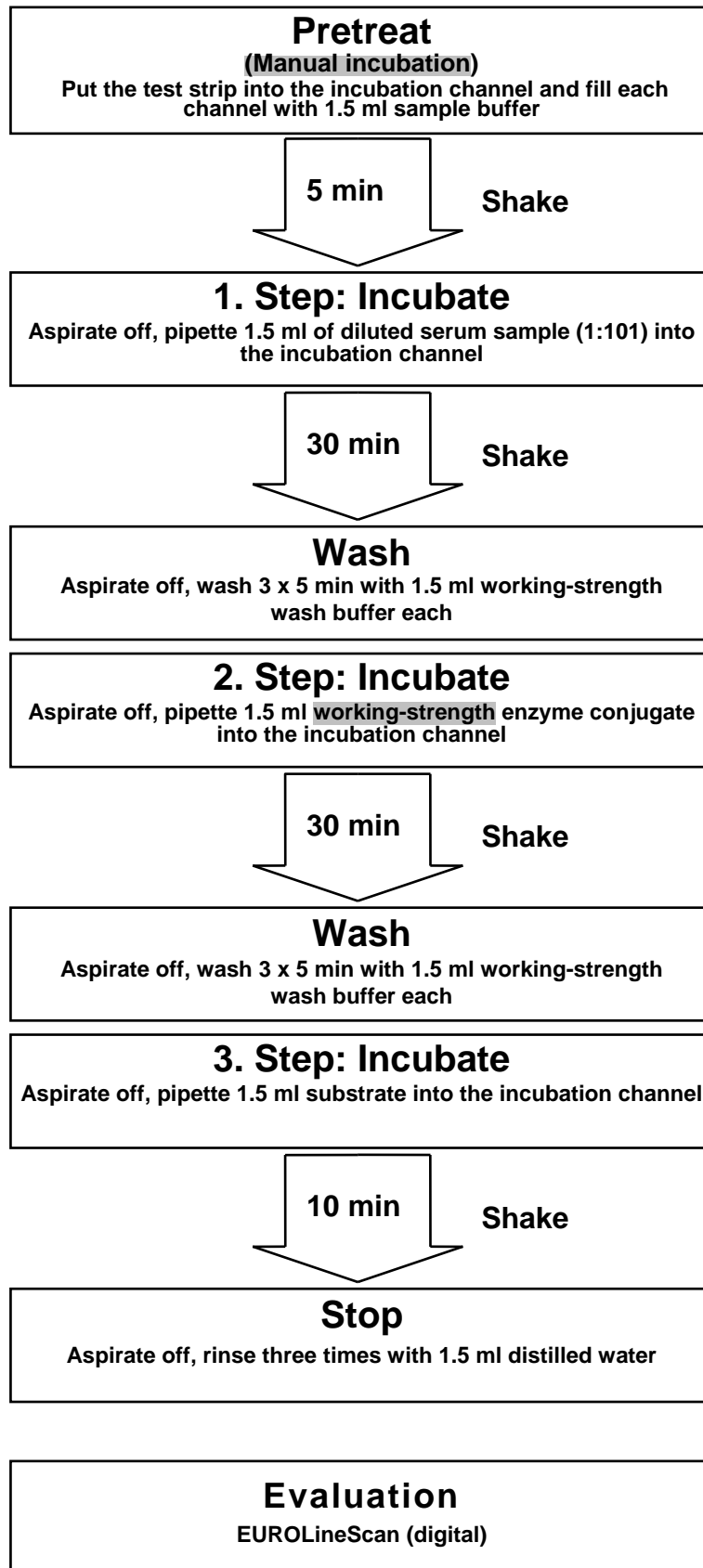
For automated incubation with the **EUROBlotOne** select the program **Euro 01/02**.

For automated incubation of Immunoblot-PreQ with the **EUROBlotOne** see instruction manual EUROBlotOne (YG_0153_A_UK_CXX).

## EUROLINE Systemic sclerosis (Nucleoli) profile (IgG)

### Incubation protocol

**Pretreat**
**(Manual incubation)**
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 working-strength 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)

# EUROIMMUN
Medizinische Laboratory Labordiagnostika AG

## 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** into EUROLineScan is **SSc profile**.

If a visual evaluation must be performed, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 1532-0101 G.

If using Immunoblot-PreQ (DL 1532-5001 G), the strips should stay in the EUROTray during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

**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 and their arrangement on the strips:** The EUROLINE test strips have been coated with the following antigens:

**Scl-70:** Scl-70 (DNA-Topoisomerase I) antigen purified by affinity chromatography from bovine and rabbit thymus.

**CENP A:** Recombinant centromere protein A. The corresponding human cDNA has been expressed with the baculovirus system in insect cells.

**CENP B:** Recombinant centromere protein B. The corresponding human cDNA has been expressed with the baculovirus system in insect cells.

**RP11:** Recombinant subunit POLR3K of human RNA Polymerase III. The corresponding human cDNA has been expressed in E. coli.

**RP155:** Recombinant subunit POLR3A of human RNA Polymerase III. The corresponding human cDNA has been expressed in E. coli.

**Fibrillarin:** Recombinant fibrillarin. The corresponding human cDNA has been expressed in E. coli.

**NOR90:** Recombinant NOR90. The corresponding human cDNA has been expressed in E. coli.

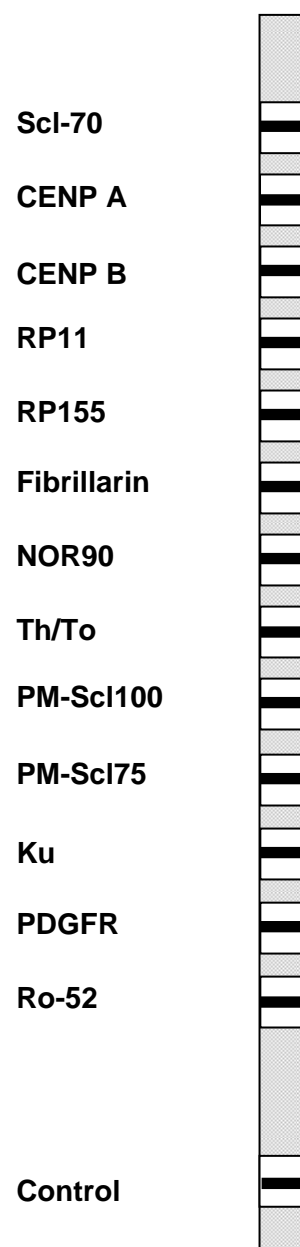**Th/To:** Recombinant Th/To. The corresponding human cDNA has been expressed in E. coli.

**PM-Scl100:** Recombinant PM-Scl protein (100 kDa). The corresponding human cDNA has been expressed with the baculosvirus system in insect cells.

**PM-Scl75:** Recombinant PM-Scl protein (75 kDa). The corresponding human cDNA has been expressed with the baculovirus system in insect cells.

**Ku:** Recominant Ku protein. The corresponding human cDNA has been expressed with the baculovirus system in insect cells.

**PDGFR:** Recombinant PDGF receptor: The corresponding human cDNA has been expressed in mammalian cells.

**Ro-52:** Recombinant Ro-52 (52 kDa). The corresponding human cDNA has been expressed with the baculovirus system in insect cells.

Scl-70
CENP A
CENP B
RP11
RP155
Fibrillarin
NOR90
Th/To
PM-Scl100
PM-Scl75
Ku
PDGFR
Ro-52
Control

EUROIMMUN recommends interpreting results based on the signal intensity:

| Signal<br>Visual evaluation | Signal intensity<br>EUROLineScan<br>Flatbed scanner | Result | |
|---|---|---|---|
| No signal | 0-5 | o | Negative |
| Very weak band | 6-10 | (+) | Borderline |
| Medium to strong band | 11-25 or 26-50 | +, ++ | Positive |
| Very strong band with an intensity comparable to the control band. | >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.
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.

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

**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 Scl-70, CENP A, CENP B, RP11 and RP155 (RNA Polymerase III subunits), Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR (platelet derived growth factor receptor) and Ro-52. 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:** 129 sera from patients with clinically characterised 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), Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR (platelet derived growth factor receptor) and Ro-52 with the EUROLINE Systemic sclerosis (Nucleoli) profile (IgG). Reaction intensities were automatically evaluated using the computer program EUROLineScan (EUROIMMUN AG). Sensitivity and specificity were calculated by ROC analysis at the given cut off value of 10 intensity units of the EUROLINEScan program [13]. Antibodies against Ro-52 are not disease-specific.

| 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 |
| **Fibrillarin** | 1.6 | 100.0 |
| **NOR90** | 3.9 | 99.0 |
| **Th/To** | 6.2 | 98.0 |
| **PM-Scl100** | 6.6 | 99.0 |
| **PM-Scl75** | 11.8 | 98.0 |
| **Ku** | 5.7 | 99.0 |
| **PDGFR** | 0.8 | 99.5 |

In 85.3% of the SSc sera, antibodies against at least one of the 12 relevant SSc antigens were detected. ROC analysis of the antibody response against each single antigen demonstrated specificities of at least 98%. The ascertained prevalences are within the range of 0.8% (PDGFR) and 65.1% for the main target antigen Scl-70. Antibodies against Ro-52 are not systemic sclerosis specific but often co-occur with antibodies against SSc specific antigens.

Furthermore the 129 sera from patients with clinically characterised SSc (diffuse and limited form) and 142 sera from the disease controls (50 patients with dermato/polymyositis, 50 with systemic lupus erythematosus and 42 with rheumatoid arthritis) were investigated with indirect immunofluorescence (IFA) using HEp-2 cells (EUROIMMUN AG) and the fluorescence patterns were correlated with the antibodies found with the EUROLINE. 99.2% (128/129) of the sera displayed an IFA positive reaction: 97 nucleolar, 12 centromere, 2 overlap (nucleolar/centromere) and 17 other patterns. The EUROLINE confirmed 93.65% (102/109) of the SSc mono-specific patterns: 100% (12/12) of the centromere and 92.8% (90/97) of the nucleolar patterns.

92.8 % (90/97) of SSc sera with a nucleolar pattern reacted positively in the SSc Profile. In contrast only 17.6% (3/17) of sera with a nucleolar pattern from the control panels were evaluated as positive. Differentiation of SSc-specific/non-specific nucleolar patterns is possible.

| Antibodies against | IFA Pattern (SSc patients n = 129) | | | | | Controls (n = 142) |
|---|---|---|---|---|---|---|
| | Nucleolar | Centromere | Nucleolar / centromere | Others | Negative | Nucleolar |
| | n = 97 | n = 12 | n = 2 | n = 17 | n = 1 | n = 17 |
| **Scl-70** | 80 | - | 1 | 2 | - | - |
| **CENP-A** | 1 | 12 | 1 | - | - | - |
| **CENP-B** | 1 | 12 | 1 | - | - | - |
| **RP11** | 6 | - | - | - | - | - |
| **RP155** | 7 | 1 | - | - | - | - |
| **Fibrillarin** | 1 | - | - | - | - | - |
| **NOR90** | 4 | - | - | - | - | - |
| **Th/To** | 8 | - | - | - | - | 1 |
| **PM-Scl100** | 5 | - | - | 3 | - | 2 |
| **PM-Scl75** | 12 | 1 | - | 4 | - | 1 |
| **Ku** | 4 | - | - | - | - | - |
| **PDGFR** | 0 | - | 1 | - | - | - |
| **Positive for at least 1 SSc antigen** | 90 | 12 | 1 | 7 | 0 | 3* |

* Sera originated from the dermato/polymyositis cohort

**Antibodies against Ro-52:** Sera from 591 patients with rheumatic autoimmune diseases, from 260 patients with autoimmune and infectious liver diseases and from 50 healthy blood donors were tested for antibodies against Ro-52 using EUROLINE. Antibodies against Ro-52 are not associated with a specific disease, but they can be found in both autoimmune and infectious diseases with a prevalence of 5% to 81% [14].

| Disease | Prevalence of antibodies against Ro-52 | |
| --- | --- | --- |
| | Serum samples | Anti-Ro52 positive (%) |
| Sjögren´s syndrome | 88 | 81 |
| Systemic sclerosis | 81 | 28 |
| Myositis | 26 | 31 |
| SLE | 210 | 38 |
| MCTD | 21 | 19 |
| Rheumatoid arthritis | 165 | 5 |
| Primary biliary cholangitis | 100 | 27 |
| Autoimmune hepatitis | 60 | 35 |
| Hepatitis B | 50 | 10 |
| Hepatitis C | 50 | 22 |
| Healthy blood donors | 50 | 0 |

## Clinical significance

For the specific serological diagnosis of the autoimmune disease systemic sclerosis (systemic scleroderma, SSc) an innovative line blot-based test combination (EUROLINE) has been designed, which is of highest diagnostic value. The test complex is based on the following autoantigens: Scl-70, CENP A and CENP B, RNA polymerase III, fibrillarin, NOR90, Th/To, PM-Scl, Ku, Ro-52, and PDGFR (platelet derived growth factor receptor). Currently, known specific autoantibodies can be found in over 95% of patients with systemic sclerosis.

Systemic sclerosis (SSc) belongs to the collagenoses, a group of autoimmune connective tissue diseases which affect the skin and internal organs. Approximately 2 to 50 of 100,000 people suffer from SSc worldwide (USA: 25 of 100,000). The incidence amounts to 1 to 2 new cases per 100,000 people per year. The disease occurs mainly in middle adulthood. Women are affected three to four times more frequently than men. The risk of acquiring the disease is particularly high among the black population. A higher frequency among members of one family is rare.

Shortening of the lingual frenum and Raynaud's syndrome (stage 1: ischaemia of the hands and feet with numbness and pain, stage 2: local cyanosis caused by hypoxia, stage 3: reactive hyperemia with redness, prickling and throbbing) are early symptoms of SSc. In the following phase oedema of the hands and feet develops. 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, leading to microstomia (reduced capability of opening the mouth) and problems in closing the eyelids. Finally, callosity of the inner organs, particularly of the digestive tract, lungs, heart and kidneys occurs.

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. The so-called CREST syndrome with calcinosis, Raynaud's syndrome, esophageal dysfunction, sclerodactyly (thin, pale, thickened and hairless skin on the fingers) and teleangiectasias (persisting pathological dilation of superficial skin vessels) is a special subform of SSc. The connective tissue of lungs, kidneys, oesophagus and heart is particularly at risk. 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%.

The diagnostic criteria for SSc established by the American College of Rheumatology (ACR) in 1980 (updated in 2007) are:

Major criterion:
- typical systemic sclerosis-like skin changes proximal to the finger basal joints

Minor criterion:
- sclerodactyly
- digital pitting scars or loss of substance from the soft parts of distal fingers and/or toes
- bilateral basal lung fibrosis

Clinical diagnosis can be established with reasonable certainty if the major criterion is present or if at least two minor criteria are met. Despite recently improved testing methods, however, the criteria are not very sensitive, particularly for early diagnosis and in patients suffering from the limited form of the disease.

Since SSc presents under various forms and on different body parts and may even come to a standstill, clinical diagnosis is difficult. Genetic factors and autoimmune processes, among others stimulating autoantibodies against the receptor of the platelet derived growth factor PDGFR, have been found to be definitely connected to the disease. Serological diagnosis is of particular importance due to the complexity of the disease. The blot test combination (EUROLINE) contains all of the main specific autoantigens known today, thus enabling a reliable serological diagnosis of SSc and simultaneously aiding in differential diagnosis. In approx. 90% of SSc cases antinuclear antibodies (ANA) can be found. The prevalences of autoantibodies against SSc-specific autoantigens are given in the table below.

| SSc-specific autoantigens | Autoantibody prevalence |
|---|---|
| **Scl-70** (DNA topoisomerase I) | Depending on activity, course and prognosis<br>**40-78%** in SSc (diffuse form)<br>**5-15%** in SSc (limited form) |
| **CENP A and CENP B** (centromere protein A and centromere protein B) | **5-10%** in SSc (diffuse form)<br>**80-95%** in SSc (limited form) |
| **RNA Polymerase III** | **5-22%** in SSc (diffuse form) |
| **Fibrillarin** (U3-RNP) | **5-10%** in SSc (diffuse form) |
| **NOR90** (nucleolus-organising region) | rarely (**<5%** in SSc) |
| **Th/To** (7-2-RNP/7-2-RNA protein complex) | rarely (**<5%** in SSc) |
| **PM-Scl** (antigen complex of 11-16 polypeptides, major antigens PM-Scl100 and PM-Scl75), incl. overlap syndrome | **10-20%** in SSc |
| **Ku** | rarely (**<5%** in SSc) |
| **PDGFR** (platelet derived growth factor receptor) | rarely (**<5%** in SSc) |

The determination of SSc-specific autoantibodies is an important element in the serological diagnosis of systemic sclerosis, including early diagnosis, activity of the disease, monitoring of the disease course, prognosis and differentiation from other collagenoses (e.g. SLE, polymyositis, Sharp syndrome and Sjögren's syndrome).

# Literature references

1. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avvedimento EV, Gabrielli A. **Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis.** N Engl J Med 354 (2006) 2667-2676.

2. EUROIMMUN AG. Janssen A, Meyer W, Scheper T, Rosemann A, Stöcker W, Schlumberger W. **Line immunoassay using two RNA-Polymerase-III subunits for the sensitive and specific detection of Systemic sclerosis (SSc) specific autoantibodies.** 7. International Congress on Autoimmunity, Ljubljana, Slowenien (2010).

3. EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. **Alle Beiträge zum Thema Autoimmundiagnostik.** In: Gressner A, Arndt T (Hrsg.) Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).

4. Hanke K, Brückner* CS, Becker M, Meyer* W, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Anti-CENP-A and anti-CENP-B antibodies show high concordance and similar clinical associations in patients with systemic sclerosis despite completely different underlying protein sequences.** (*EUROIMMUN AG) In: Conrad K et al. (Hrsg.). From Pathogenesis to Therapy of Autoimmune diseases: Autoantigens, Autoantibodies, Autoimmunity. Pabst Science Publishers (2009) 392-393.

5. Hanke K, Brückner* CS, Dähnrich* C, Huscher D, Becker M, Komorowski* L, Meyer* W, Jansen* A, Backhaus M, Becker M, Kill A, Egerer K, Burmester G, Hiepe F, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients.** Arthritis Research & Therapy 11(1): R22 (2009).

6. Hanke K, Janssen* A, Meyer* W, Scheper* T, Komorowski* L, Brückner* C, Egerer K, Hiepe F, Riemekasten G. (*EUROIMMUN AG). **Prevalence of autoantibodies against PM/Scl75 and PM/Scl100 in systemic sclerosis.** 10. International Workshop on Autoantibodies and Autoimmunity (IWAA), Mexiko (2008).

7. Hanke K, Uibel S, Brückner* C, Dähnrich* C, Egerer K, Hiepe F, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Antibodies to CENP-B antigen identify a subgroup of systemic sclerosis patients presenting more frequently sicca syndrome and less frequently lung fibrosis, cardiac and vascular involvement – analysis of the Charité SSc cohort.** In: Conrad K et al. (Hrsg.). From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies. Pabst Science Publishers 5 (2007) 477-478.

8. Ho KT, Reveille JD. **The clinical relevance of autoantibodies in scleroderma.** Arthritis Res Ther 5 (2003) 80-93.

9. Jarzabek-Chorzelska M, Meyer* W, Blaszczyk M, Kolacinska-Strasz Z, Teegen* B, Jablonska S. (*EUROIMMUN AG). **Scl-70 antibodies are highly specific and prevalent in systemic scleroderma (SSc).** In: Conrad K et al. (Hrsg): Autoantigens and Autoantibodies: Diagnostic Tools and Clues to Understanding Autoimmunity. Pabst Science Publishers 1 (2000) 706.

10. Jarzabek-Chorzelska M, Meyer* W, Blaszczyk M, Kolacinska-Strasz Z, Teegen* B, Jablonska S. (*EUROIMMUN AG). **Scl-70 antibodies are highly specific and prevalent in systemic scleroderma (SSc).** In: Conrad K et al. (Hrsg): Autoantigens and Autoantibodies: Diagnostic Tools and Clues to Understanding Autoimmunity. Pabst Science Publishers 1 (2000) 706.

11. Mahler M, Raijmakers R, Dähnrich* C, Blüthner M, Fritzler MJ. (*EUROIMMUN AG). **Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen.** Arthritis Res Ther 7 (2005) 704-713.

12. Mahler M, Siegemund* M, Meyer* W, Petschinka M, Fritzler MJ. (*EUROIMMUN AG). **Anti-PM/Scl-antibodies in systemic sclerosis: Comparison between PM1-Alpha ELISA and a line assay with recombinant PM/Scl-75c and PM/Scl-100.** In: Conrad K et al. (Hrsg.). From Pathogenesis to Therapy of Autoimmune diseases: Autoantigens, Autoantibodies, Autoimmunity. Pabst Science Publishers (2009) 398-399.

13. Meyer* W, Janssen* A, Vencovsky J, Putova I, Becvar R, Scheper* T, Komorowski* L, Probst* C, Stoecker* W, Schlumberger* W. (*EUROIMMUN AG). **A new line blot immunoassay for the parallel detection of 12 systemic sclerosis (SSc) specific autoantibodies.** Eingereicht als wissenschaftliche Präsentation (Poster) zum 7. International Congress on Autoimmunity, Ljubljana, Slowenien 5.-9. Mai 2010.

14. Meyer* W, Scheper* T, Janssen* A, Siegemund* M, Chen S, Rosemann* A, Schlumberger* W, Stöcker* W. (*EUROIMMUN AG). **Prevalence of antibodies against Ro-52 in various rheumatic autoimmune diseases, primary biliary liver cirrhosis and autoimmune and infectious hepatitis.** Annals of Rheumatic Diseases 67 Suppl. II (2008)146.

15. Schlumberger* W, Meyer* W, Proost* S, Dähnrich* C, Müller-Kunert* E, Sonnenberg K, Olbrich S, Stöcker* W. **Die neue EUROBLOT-Technologie: Differenzierung der Autoantikörper gegen Zellkerne. (*EUROIMMUN AG).** Poster zur Tagung Biochemische Analytik 95, 25. bis 28. April 1995 in Leipzig. Abstrakt im Kongressband (1995).

16. Uibel S, Hanke K, Dähnrich* C, Brückner* C, Egerer K, Hiepe F, Schlumberger* W, Riemekasten G. (*EUROIMMUN AG). **Anti-Scl70 antibody levels correlate with skin and organ fibrosis in patients with systemic sclerosis – analysis from the Charité SSc cohort.** (*EUROIMMUN AG) In: Conrad K et al. (Hrsg). From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies. Pabst Science Publishers 5 (2007) 475-476.

17. Varga J. **Systemic sclerosis: an update.** Bull NYU Hosp Jt Dis 66 (2008) 198-202.